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**AUTONOMIC NERVOUS
INVOLVEMENT
IN STRESS-INDUCED ACTH
SECRETION**

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P. G. SMELIK

AUTONOMIC NERVOUS INVOLVEMENT IN
STRESS-INDUCED ACTH SECRETION

N.V. DRUKKERIJ V/H H. BORN - ASSEN

RIJKSUNIVERSITEIT TE GRONINGEN

AUTONOMIC NERVOUS INVOLVEMENT IN STRESS-INDUCED ACTH SECRETION

A STUDY ON THE PERIPHERAL AND DIENCEPHALIC
AUTONOMIC INFLUENCES UPON THE REGULATION OF THE
ADRENOCORTICOTROPHIC ACTIVITY OF THE
PITUITARY GLAND IN RATS



PROEFSCHRIFT

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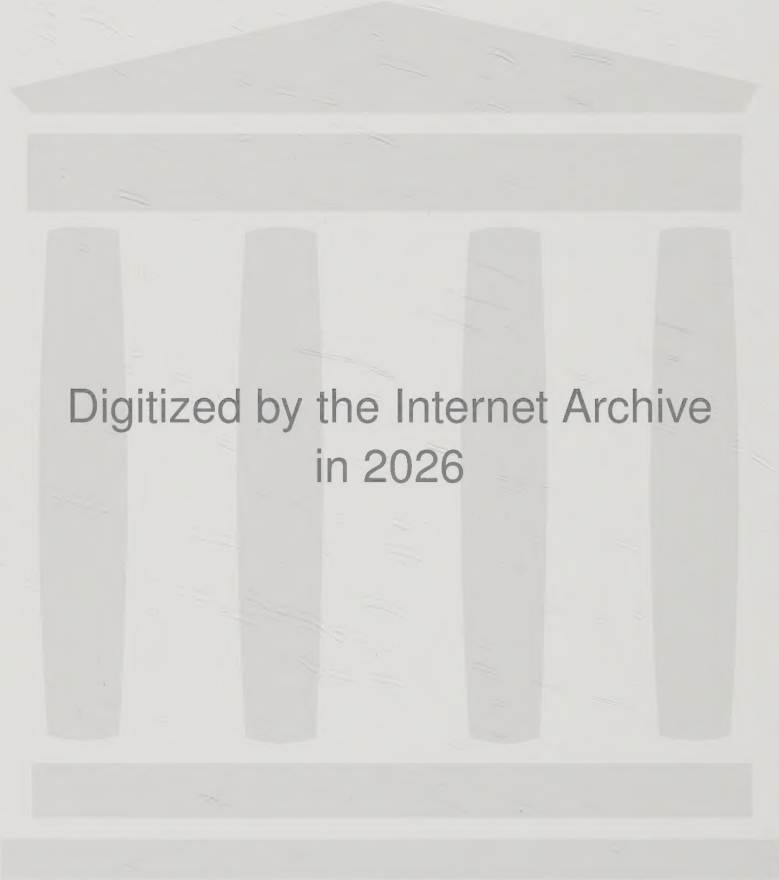
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Aan mijn Ouders



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GENERAL INTRODUCTION

During the last years the regulation of the activity of the adrenal cortex has been the main topic of experimental work in this laboratory. Since the function of the adrenocortical hormones is placed within the framework of the so-called stress phenomena, more attention has been paid to the mechanism through which it is controlled during stress. As it is known that the production and secretion of the corticoids is regulated by the adrenocorticotrophic hormone (ACTH) from the pituitary gland, the problem has been shifted to the question: which factors govern the hypophysial secretion of ACTH due to stress?

“Stress” may be shortly defined as the resistance of the organism opposed to unspecific damage. There are numerous “stressor stimuli” which cause an enhanced ACTH secretion. Analysis of the factors operating in the controlling mechanism of the hypophysial corticotrophic function should not eliminate the role of the autonomic nervous system in this respect. The important part of this system played in defense reactions of the higher vertebrates cannot be doubted since CANNON’s concept of homeostasis.

The object of this study, therefore, is the cooperation and interaction between the regulation of the hypophysial corticotrophic activity and the autonomic nervous system during stress. The description of our experimental work will be preceded by a review of different theories concerning the defense mechanism operating and the autonomic functions during stress. As the most important concepts mainly have covered their own geographical sphere of influence sometimes even without being aware of other analogous theories, an attempt is made to offer a comparative consideration of these views. In our opinion this is a necessary condition for an objective experimental approach of the endocrine functions in adaptive and homeostatic reactions of the mammalian organism.

REGULATION OF ADAPTIVE MECHANISMS

Review of the pertinent literature

Imagination, one of the great powers of the mind, is not only desirable, but absolutely necessary for the advancement of science.

B. A. Houssay.

1. INTRODUCTION

The regulation of body defense mechanisms can be considered from different standpoints. Since defense is a general effort of the organism as a whole, every organic system participates in this reaction, e.g. the nervous systems, endocrine system, heart and blood circulation, respiratory system, gastrointestinal system, muscular system, reticulo-endothelial system, etc. Is it possible to designate one site, one organ or one system which would be responsible for the regulation and integration of the adaptative reactions? At least one is intended to award this role to the nervous and/or the endocrine systems, for these seem designed for the control of the organic functions. Indeed, most attention has been focused on these controlling systems. We shall therefore review the literature on the role of those systems in adaptation processes: the endocrines, the autonomic nervous system and the central nervous system.

2. THE ENDOCRINE VIEW

A. *The Adaptation Syndrome of Selye*

The name of SELYE is attached to the elucidation of the role played by hormones in the adaptative reactions. Undoubtedly it is his merit to have drawn attention to the adaptive function of the adrenocorticosteroids, and to have afforded a general and comprehensive view of adaptive phenomena.

The story of his "Adaptation Syndrome" started in 1936, when he happened to find a new syndrome due to the administration of certain crude ovarian extracts (SELYE 1952). This syndrome consisted of three phenomena: adrenal enlargement, thymicolymphatic involution and gastrointestinal ulcers. Initially he thought that this syndrome was due to a new humoral factor, which consequently now was to be purified. However, it turned out that not only purer preparations were less active, but also crude extracts of other organs produced the same syndrome. Reasoning that the mere toxicity of all these impure extracts might account for this effect, he injected a damaging agent like formalin, which also appeared to elicit the triad of manifestations even clearer than before.

Nevertheless, it seems that a scientific worker always should be endowed with an extremely supple mind in considering his experimental results, especially when they are negative. It suddenly occurred to SELYE that this syndrome of response to "damage as such" might be a general unspecific reaction to all pathological disturbances, and that any disease and any noxious agent would produce this unspecific syndrome together with its specific actions. If this were true, the aetiology of much suffering perhaps could be traced and in this way combatted.

From these ideas the concept of the General Adaptation Syndrome (G-A-S) was developed. A detailed review of this concept has been published in 1950. Each following year an Annual Report on Stress has been published, containing new facts and amendments (SELYE 1950, 1951, SELYE & HORAVA 1952, 1953, SELYE & HEUSER 1954, 1956). A review of this concept and its development is given schematically.

The G-A-S is the sum of all non-specific systemic reactions of the body which ensue upon continued exposure to stress. This condition of stress is elicited by stressor stimuli, which show a wide-spread diversity: e.g. trauma, heat, cold, burns, haemorrhage, X-rays, toxic drugs, overdosage of hormones, muscular work, anoxia, nervous and emotional stimuli. The stress itself was initially defined as the condition resulting from a damaging force and the resistance opposed to it. Later on, the term is used to denote "the sum of all bodily changes caused by function or damage", which become manifest in the G-A-S (SELYE & HEUSER 1956). This definition is not very satisfactory, for the difference between "stress" and G-A-S is now rather indistinct.

The G-A-S comprises three stages, namely the Alarm-Reaction (A-R), the Stage of Resistance (S-R) and the Stage of Exhaustion (S-E).

Originally the A-R was characterized by the syndrome of adrenal enlargement, involution of the thymus and lymphatic tissues, and gastrointestinal ulcers, but further analysis revealed a division of the A-R into two phases, shock and counter-shock. The shock phase is the early passive phase following the exposure to stress, during which the defense forces are not yet mobilized. It is characterized by hypotension, hypothermia, deranged capillary permeability, hypoglycemia, acidosis, leucopenia, eosinopenia, haemoconcentration and other phenomena. During this phase the gastrointestinal lesions occur as well. In due time manifestations of defense appear: discharge of adrenaline, ACTH, corticoids, and, for a part as a consequence of these processes, the reverse of the changes appearing in the shock phase. This counter-shock represents the effort to acquire adaptation, which is reached in the S-R. The animal is then adapted to the stressor agent, but has a decreased resistance to other stimuli. When a stress is con-

tinued, the S-R cannot be maintained indefinitely, and adaptation is lost again. This S-E, which also may occur when the S-R is not yet acquired, in the case of a severe stress, is characterized by many A-R manifestations. After having consumed all its (adaptation) energy, the animal dies from exhaustion.

Diseases of adaptation may occur due to an insufficient, excessive or abnormal adaptive reaction. As such are considered Cushing's disease, rheumatic diseases, nephroscleritis, hypertension, Simmond's disease, Addison's disease, Waterhouse-Friderichsen syndrome and other syndromes.

The analysis of the stress mechanism started from the triad of A-R manifestations. It was soon found that the thymicolymphatic changes are dependent of the presence of adrenocorticosteroids, but that gastrointestinal lesions also appear after adrenalectomy. In the mean time the corticotropic principle (ACTH) from the pituitary was discovered, and so the anterior pituitary was introduced in the stress mechanism. Stressor agents no longer stimulate the adrenal cortex in hypophysectomized animals. In 1944, it was established that desoxycorticosterone acetate (DOCA) causes many rheumatic and other damaging and inflammatory changes in various organs. This fact led to a division of the adrenocortical principles in prophlogistic and anti-phlogistic corticoids. Moreover, the somatotropic hormone (STH) appeared to exert prophlogistic effects, perhaps partly mediated through the adrenal cortex. Since 1952 new experimental results suggested that the mineralocorticoids (DOCA and the recently discovered aldosterone) mainly exert prophlogistic effects, whereas the glucocorticoids (cortisol, cortisone, hydrocortisone) are said to be anti-phlogistic. It is not yet settled if one ACTH enhances both the glucocorticoid and the mineralocorticoid production, and if STH stimulates the secretion of mineralocorticoids or directly sensitizes the peripheral tissues to them.

It is of importance that SELYE admits that in hypophysectomized animals, i.e. in the absence of ACTH, STH and corticoids, the three stages of the G-A-S do occur. The adaptive hormones act principally by facilitating the acquisition of adaptation and by prolonging the stage of resistance (SELYE & HORAVA 1952, p. 27).

An interesting problem concerns the question of the so-called First Mediator. Up to now it is not yet known how the stressor stimulus reaches the hypophysis and influences other tissues (as the gastrointestinal tract) directly. In the numerous schematic drawings in SELYE's Reports on Stress an interrupted arrow, decorated with a question mark, indicates the ignorance of the way in which the damage is informed to the defense mechanism. "We still know virtually nothing about the nature of the "first mediator(s)"

which transmit(s) the impulse for ACTH secretion from the area directly affected by stressors . . ." (SELYE & HEUSER 1956) (See Fig. 1).

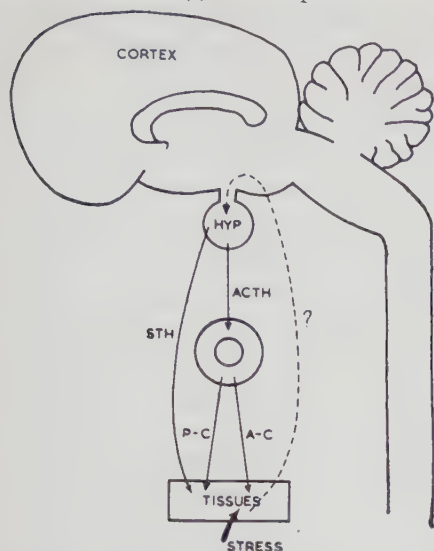


Figure 1.

Principal pathways of nonspecific effects upon the target according to SELYE & HEUSER 1956).

Perhaps a corner of the veil is lifted by the recent establishment that the hypothalamus is involved in the regulation of the ACTH secretion. Consequently the problem of the First Mediator has been shifted to the question: How reaches the stimulus the hypothalamus? The chain of causal relations of the stress mechanism apparently would be one link longer. Nevertheless, in this hormonal pattern of stress reactions the occurrence of a nervous centre in the mechanism is significant. We shall therefore discuss now some aspects of the ACTH regulation in detail.

B. Humoral feed-back

A simple mechanism for the regulation of the ACTH secretion was proposed by SAYERS & SAYERS (1948), who advanced a humoral feed-back theory. They showed that adrenal cortical extracts prevented the ACTH release, when injected prior to the application of a stress. Exogenous ACTH injected in animals treated with cortical extracts causes a normal adrenocortical activation (SAYERS & SAYERS 1947). They demonstrated a quantitative relationship between the degree of inhibition of ACTH secretion and the amount of administered cortical hormones, and also between the intensity of the stress and the required amount of corticoids for inhibition of the ACTH release. As the utilization of cortical hormones during stress by the peripheral tissues was assumed to be enormously enhanced, the circulating amount of these hormones would be decreased. The decreased blood level of corticoids would stimulate the hypophysis to secrete ACTH in larger amounts, thus enhancing the production of corticoids. When the need for cortical hormones has come to an end, either by removal of the stressor stimulus or by acquisition of resistance, the increasing titer of corticoids will inhibit progressively the ACTH production (Fig. 2).

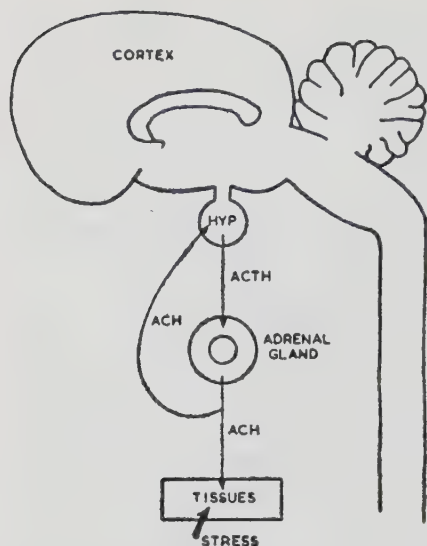


Figure 2.

Scheme representing the view of SAYERS (1948) on the regulation of the ACTH secretion by the systemic blood level of adrenocortical hormones.

Further research did not corroborate this attractive theory. It was shown in the subsequent years that the time interval required for this feed-back mechanism is too long to account for the increased ACTH secretion following stress. GRAY & MUNSON (1951) showed that ACTH is discharged within ten seconds following the intravenous injection of histamine. Direct measurement of the ACTH content of blood samples, taken immediately after the application of a stressful stimulus, indicated that this content is significantly increased within two minutes (SYDNOR & SAYERS 1954). It is almost impossible that during this short period the enhanced peripheral utilization of corticoids already would have lowered the corticoid blood level.

In addition, there is no proof that the tissue utilization of corticoids actually increases by stress (COWIE *et al.* 1954, ULRICH & LONG 1956, EIK-NES & SAMUELS 1958). An artificial decrease of the corticoid titer by dilution of the blood perfusing the pituitary gland did not enhance the ACTH secretion (BUSH *et al.* 1953). BARRETT & HODGES (1956) found that blood of adrenalectomized rats did not contain more ACTH as blood derived from normal rats. BRODISH & LONG (1956) and COX *et al.* (1958) found that after bilateral adrenalectomy in rats the blood level of ACTH rapidly decreases and is restored to normal only several days afterwards, and then slowly further increases to supra-normal levels.

Concerning the initiation of the ACTH secretion another mechanism must be supposed. SYDNOR & SAYERS (1954) assumed that a hypothalamic factor may cause the rapid discharge of ACTH (neural mechanism), whereas the subsequent rate of discharge is regulated by the titer of corticosteroids (humoral mechanism). Apart from the newer evidence that the corticoid titer only may exert an inhibiting influence, the differentiation between a hypothalamic neural mechanism and a peripheral humoral mechanism seems also to be unjustified. Recent investigations suggest that the site of action of the corticoids is, at least partly, the brain stem. Venous brain blood obtained from stressed hypophysectomized rats causes an eosino-

nopenia when injected in rats in which the hypothalamus was destroyed, thus indicating that the donor blood contained a hypothalamic factor activating the anterior pituitary of the recipient rat for ACTH secretion.¹⁾ If the donor rats were pretreated with DOCA, no eosinopenia occurred in the recipients (SCHAPIRO *et al.* 1956, 1958). Analogous findings are reported by PORTER & JONES (1956), who showed that hypophyseal portal vessel blood obtained from the severed pituitary stalk in hypophysectomized dogs, elicits an adrenal ascorbic acid decrease in intact rats pretreated with hydrocortisone. Other reports do not corroborate this view. GANONG & HUME (1955) concluded from experiments on the influence of hydrocortisone on the compensatory adrenal hypertrophy in the dog that the inhibiting effect of corticoids on the ACTH secretion is not influenced by lesions destroying the median eminence of the tuber cinereum. ROSE & NELSON (1956) using a micro-injector technique to deliver hydrocortisone directly to the hypophyseal fossa of rats, found that hydrocortisone produced an ACTH inhibitory effect.

Quite recently McCANN *et al.* (1958) reported that in rats bearing hypothalamic lesions which reduced the compensatory adrenal hypertrophy after unilateral adrenalectomy, a further inhibition of this hypertrophy could be obtained by daily administration of hydrocortisone. They assumed therefore, that corticoids suppress both the release of the CRF and the excitability of the hypophysis itself.

This evidence is not convincing, since their hypothalamic lesions exerted only a partial inhibition of the compensatory adrenal hypertrophy, whereas in our experience median eminence lesions completely inhibit the hypertrophy of the remaining adrenal gland (DEWIED *et al.* 1957). It is likely that in McCANN's experiments the production of the CRF was not entirely inhibited, and that consequently hydrocortisone caused a further block of the CRF-release.

PORTER (1953) observed an enhanced electrical activity of the anterior brain stem following the injection of adrenal cortical extract. The ability of steroids to induce anesthesia in high doses also suggests that they suppress brain stem structures, presumably the reticular substance.

If the endogenous corticosteroids inhibit the ACTH release in stressful conditions, is not yet certain. The amounts necessary for this inhibition are rather high and perhaps on a supra-physiological level. Since it is very likely that the peripheral utilization of the corticoids is not enhanced during stress, a much higher level will be present than was assumed formerly. The physiological significance of such an inhibition is not yet clear, since

¹⁾ For this substance (CRF) see p. 19.

BRODISH & LONG (1956) showed that the ACTH production is completely inhibited a few hours after unilateral and bilateral adrenalectomy. In this case the enhanced production of corticoids cannot be the cause of the hypophysial inhibition.

At any rate it seems plausible to assume that an inhibiting influence of the cortical steroids exists, and that this effect is at least partly mediated through the hypothalamus.

C. Adrenergic Mediation

It was recognized rather early that adrenaline might play an important role in the regulation of ACTH secretion. Injection of adrenaline causes an increased activity of the adrenal cortex as measured on the adrenal ascorbic acid and cholesterol depletion, but not after hypophysectomy (LONG & FRY 1945). Intramuscular infusion of as little as 0.5—2 γ adrenaline during one hour in nembutalized rats caused an ascorbic acid and cholesterol depletion as well as an eosinopenia (GERSHBERG *et al.* 1950). The effect of various stressor agents which were known to cause an eosinopenic response in normal rats within one hour, was abolished after interruption of the autonomic efferent pathways from the midbrain towards the adrenal medulla. These interruptions consisted of diencephalic lesions, spinal section at the third thoracic vertebra level or adrenal demedullation.

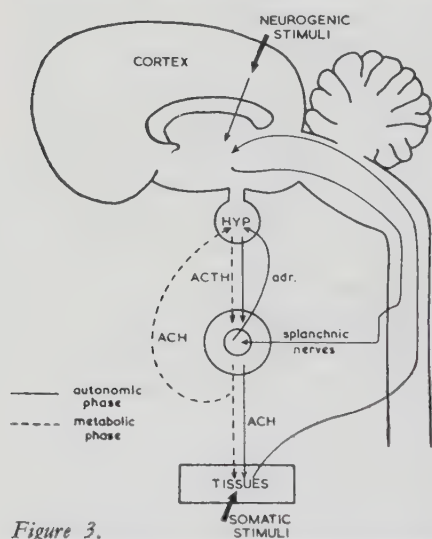


Figure 3.
Scheme representing the view of LONG on the regulation of the ACTH secretion by the adrenomedullary and adrenocortical hormones (after McDERMOTT *et al.* 1950).

In these cases the eosinopenic response is blocked at the first hours following stress, but occurs as normal at the fourth hour (McDERMOTT *et al.* 1950).

These facts support the hypothesis advanced by LONG (1947), that the secretion of ACTH is regulated by a dual mechanism, in which reflex adrenaline would stimulate the pituitary directly during the first hour ("autonomic phase") and a humoral mechanism as proposed by SAYERS & SAYERS (1948) would be responsible for the subsequent "metabolic phase". During the autonomic phase the stressor stimulus would cause the release of adrenaline from the adrenal medulla via the sympathetic trunk and splanchnic nerves (Fig. 3).

This view is very attractive in this

respect that the role of the autonomic nervous system and of adrenaline in emergency, which had been already emphasized by CANNON (1928) is given an important position in the frame of the hypophyseal stress response.

Attempts to prevent the ACTH discharge with adrenolytic drugs initially were indecisive, since these agents act as a stimulus for ACTH secretion. However, SAWYER & PARKERSON (1953) showed a complete blockade of the eosinopenia 1 hour after the injection of adrenaline, when the animal was pretreated with the dibenamine analogue SKF-501 24 hours previously. Similar results were obtained by LOUWERENS & SMELIK (1953), who demonstrated that hexamethonium, a ganglion-blocking agent which does not stimulate the hypophyseal-adrenal axis, abolished the "one-hour eosinopenia" following a painful stimulus.

Nevertheless, the absence of the eosinopenic response after one hour does not prove that no release of ACTH had occurred. The eosinophil test is probably not sensitive enough to indicate a slightly elevated ACTH content in the blood. Most authors agree that the ACTH secretion is not abolished, but at most diminished when the release of endogenous adrenaline is prevented. VOGT (1951) showed that the adrenal ascorbic acid depletion in adrenal demedullated rats to an emotional stimulus was reduced as compared with the response in normal rats. The adrenal response in demedullated rats appeared to be diminished, but not delayed. She also demonstrated that this residual release of ACTH was not due to adrenaline released from other sources (VOGT 1952). GORDON (1950a) showed that in adrenal demedullated rats the ascorbic acid depletion following the injection of adrenaline, insulin or histamine is not diminished. Fracture of a leg and a mild scald induced a greater reduction in ascorbic acid content when they were applied to the innervated than to the denervated leg. Severe scalding is not influenced by denervation, but invariably caused an ascorbic acid depletion in demedullated rats (GORDON 1950 b). He suggested that in certain types of stress as fracture or mild scald, where there is little tissue destruction, the stress is mainly neurotropic and nervous activation of the adrenal medulla may play a major role in the activation of the hypophysis. In cases of a greater degree of tissue destruction the pituitary-adrenal axis can be activated even in the absence of nervous impulses.

Although the adrenal ascorbic acid depletion is a more reliable criterion than the eosinopenia, the latter technique might provide some data which suggest that a neurogenic stress is more intimately associated with the autonomic adrenaline mechanism than a traumatic or metabolic stress. McDERMOTT *et al.* (1950) reported that in adrenal demedullated rats the

eosinophil response to laparotomy and cold is inhibited after one hour, but at the end of four hours there is a dramatic fall in the eosinophil number. In contrast, a painful stimulus is not able to change the eosinophil number during four hours in spinal sectioned rats. They concluded that a painful or emotional stimulus in the absence of severe physical stress is dependent on the reflex secretion of adrenaline, and is not mediated through any other neural or neurohumoral pathway. Accordingly, GELLHORN & FRANK (1949) found that "mild" stresses like rage and electrically induced convulsions did not cause a lymphopenia in adrenodemedullated rats, in contrast to insulin hypoglycemia.

From studies in man it appeared that emotional disturbances readily cause an adrenomedullary hormone discharge, but that in patients with a normal operative and postoperative course the adrenaline secretion is usually normal (FRANKSSON *et al.* 1954).

It is apparent that the release of adrenaline from the adrenal medulla enhances the ACTH discharge during stress, but that it cannot account for the initial stimulus in any case. This is also illustrated by the fact that histamine injected intravenously induces an ACTH release more rapidly than intravenous administration of adrenaline (GRAY & MUNSON 1951).

Nevertheless, the adrenaline mechanism may represent an important factor in the regulation of ACTH secretion during stress. The exact mechanism, however, is poorly understood. According to LONG (1952) the stress stimulus activates the hypothalamus, which in turn stimulates the adrenal medulla via efferent neural pathways. The subsequently released adrenaline would act directly on the pituitary gland since adrenaline in high doses is capable to cause an ACTH liberation from pituitary transplants in the anterior chamber of the eye (FORTIER 1951, McDERMOTT *et al.* 1950). This assumption is, however, not substantiated by investigations on the effect of hypothalamic lesions on the action of adrenaline on the ACTH secretion. It was stated repeatedly that these lesions abolished the ACTH releasing activity of adrenaline (HUME 1952, PORTER 1953, LAQUEUR *et al.* 1955, SMELIK & DEWIED 1958), which indicates that adrenaline activates hypothalamic structures.

PORTER (1952) found that intravenous injection of 10 γ adrenaline in cats produced a marked elevation in electrical activity of the posterior hypothalamus. Hypoxia and insuline had the same effect, while these stimuli caused a simultaneous eosinopenia. Lesions destroying the posterior hypothalamus prevented the eosinopenic response to adrenaline, histamine and formalin; anterior lesions were without effect. Electrical excitation of the tuberal and posterior region likewise induced an eosinopenia (PORTER 1953). These results suggest that the posterior hypothala-

mus plays an essential role in the ACTH releasing mechanism, and that adrenaline activates preferably this region (Fig. 4).

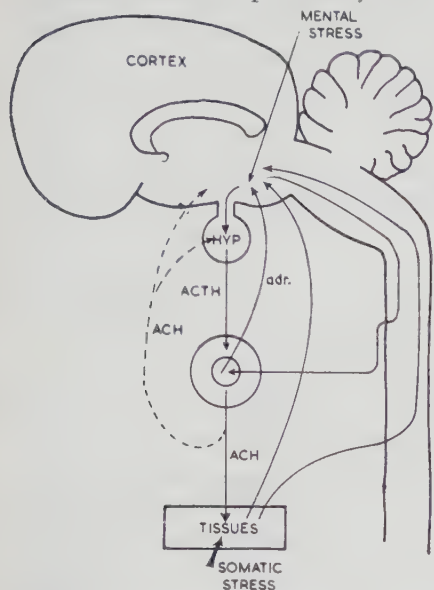


Figure 4.
Scheme representing the view of PORTER (1953) on the regulation of the ACTH secretion by the posterior hypothalamus.

sectomized and adrenalectomized rats (DEWIED, unpublished observations). Thus an extra-adrenal influence of the posterior hypothalamus on the eosinophil count may not be excluded.

In summary, the role of endogenous adrenaline in the control of ACTH secretion is not yet clear. Nevertheless, the research in these lines strongly suggested hypothalamic involvement in the ACTH releasing mechanism as was the case in the analysis of the humoral theory of SAYERS. The "autonomic" mechanism as well as the "metabolic" mechanism requires the cooperation of hypothalamic centres.

D. Direct Hypothalamic Control

Further research based upon either of the above reviewed theories strongly suggested a hypothalamic involvement in the control of pituitary function. The importance of the direct connections between hypophysis and hypothalamus as a possible pathway for the control of the hypophyseal stress response has been recognized by HARRIS (1948), who postulated that as a result of hypothalamic activation a substance is produced which

The only reasonable explanation of these experiments is that the stimulus travels directly from the posterior hypothalamus to the pituitary gland via the hypophyseal stalk. In that case the function of adrenaline remains obscure.

However, this work has met serious criticism. The usefulness of the eosinophil criterion, especially with regard to the adrenal activation by adrenaline, has been seriously doubted (ESSELLIER *et al.* 1957, cf. PORTER 1954, Discussion), and also the use of anesthesia might have caused erroneous results (see FORTIER 1956, HARRIS 1955, p. 123). Moreover, a lipide extract from bovine posterior hypothalamus prepared according to SLUSHER & ROBERTS (1954) appeared to cause an eosinopenia in hypophy-

is carried to the anterior pituitary via the portal vessels and is capable to stimulate the ACTH secretion.

The anatomical relations between the basal hypothalamus and the hypophysis are in favour of this assumption. It cannot be irrelevant that the pituitary gland is closely connected with the diencephalon through the hypophyseal stalk. Whereas abundant nervous pathways are present between hypothalamic nuclei and the neurohypophysis, a similar nerve supply of the adenohypophysis is doubted. However, the arterial blood supply of the anterior lobe is derived from a primary capillary bed, situated in the median eminence of the infundibulum. From this bed hypophyseal portal vessels convey the blood to a second capillary bed, the sinusoids of the pars distalis (XUEREB *et al.* 1954). In the rat the blood supply of the pars distalis seems to be exclusively restricted to the portal circulation (DANIEL & PRICHARD 1956). Thus, it is obvious that a hypothalamic humour can be picked up by the capillary loops in the median eminence and carried towards the anterior lobe.

The most direct evidence pertaining a humoral transmitter has been presented by PORTER & JONES (1956), who showed that extracts from plasma, obtained from the severed portal vessels in hypophysectomized dogs, cause an adrenal ascorbic acid depletion in hydrocortisone-treated rats. Further evidence is obtained by SCHAPIRO *et al.* (1956), who administered venous brain blood of stressed hypophysectomized rats into rats bearing hypothalamic lesions which had impaired the ACTH secretion. They observed a definite eosinopenic response in the recipients, whereas blood derived from non-stressed rats was inactive in this respect.

Several attempts have been made for isolation of the neurotransmitter. SLUSHER & ROBERTS (1954) were the first to claim that a lipide extract from bovine posterior hypothalamus would be capable of stimulating pituitary release of ACTH, and that this extract might represent in crude form the natural neurohumor. However, recent studies by DEWIED *et al.* (1958) showed that a similar extract is inactive in hydrocortisone-inhibited rats and in rats with hypothalamic lesions.

SAFFRAN and coworkers obtained an ACTH liberation from anterior lobe tissue *in vitro* by an extract of neurohypophyseal tissue (SAFFRAN & SCHALLY 1955). The active component, the corticotrophin releasing factor (CRF), could be separated by paper chromatography, and appeared to be distinct from any of the known posterior lobe hormones (SAFFRAN *et al.* 1955). Using a similar *in vitro* test, GUILLEMIN *et al.* (1956) reported the presence of a CRF in extracts from brain tissue and in substance P. By the paper chromatographic method an active factor DA was separated,

which appeared to be a small peptide, different from vasopressin and other known hypothalamic principles (GUILLEMIN *et al.* 1957).

However, serious objections against the *in vitro* assay are raised by several workers. SWINGLE *et al.* (1956), using the same test, reported afterwards that doubts were raised as to the validity of the test, as it appeared to be unspecific. BARRETT & SAYERS (1958) suggest that the so-called CRF's only inhibit the degradation of ACTH in the *in vitro* system. No difference between the action of extracts from serum, obtained from normal, stressed and adrenalectomized rats, on the ACTH liberation from pituitary tissue *in vitro* was observed by FORTIER & WARD (1958), which was the motive for assuming that the occurrence of ACTH in the medium may simply reflect passive leakage of the hormone from the tissue. In these critical studies, however, modifications of the original method were used. Maybe the original test is valid, provided that one is aware of its limitations as being only a model of the natural mechanism. When assayed *in vivo*, the isolated factors seem to be rather inactive. McCANN (1957) reports that Fraction D4 which he received from GUILLEMIN, was unable to cause an adrenal response in hypothalamic lesioned rats. On the other hand, CLAYTON *et al.* (1958) obtained a rise in 17-hydroxy-corticoids in plasma of children within 15 minutes after intravenous injections of Fraction D4; inactivated fractions had no influence on the 17-OH-Cs level.

There are many claims that the CRF is identical with the posterior lobe hormone vasopressin. For this assumption the following circumstantial evidence exists:

- a. the capillary loops of the primary plexus in the median eminence are in close contact with the nerves of the supraoptico-hypophysial tract, which carries the posterior lobe hormones downwards as neurosecretory material (PALAY 1953; SCHARRER & SCHARRER 1954);
- b. the neurosecretory material is readily mobilized into the portal vessels following the application of stress (ROTHBALLER 1953, 1956);
- c. stressful situations cause an immediate rise in plasma antidiuretic activity correlating with the release of ACTH (MIRSKY *et al.* 1954 a, b);
- d. the commercial vasopressin-preparation Pitressin causes an ACTH liberation from the hypophysis in hydrocortisone-inhibited and hypothalamic-lesioned animals (McCANN & BROBECK 1954; McCANN 1957; DEWIED *et al.* 1958).

McCANN & FRUIT (1957) reported that synthesized lysine-vasopressin (DuVIGNEAUD) is highly active in lesioned rats, with a greater potency than Pitressin. It is known that lysine-vasopressin is two times as potent as arginine-vasopressin (McDONALD & WEISE 1956).

In summary, there can be little doubt that lysine-vasopressin and closely-related polypeptides may exert CRF-activity, but exact knowledge of the nature of these principles and their releasing mechanism is still lacking.

Concerning the site of the CRF-production, no definite results are obtained. The data derived from hypothalamic stimulation and coagulation are discussed in Chapter III. There is now general agreement that the median eminence must be destroyed in order to obtain a successful inhibition of the hypophyseal stress response. As it is unlikely that the median eminence itself would represent a transmitter-producing structure, the effectiveness of these lesions are to be sought in the interruption of the pathways leading to the hypophysis (SMELIK *et al.* 1959). Nevertheless, experiments with animals in which the median eminence is destroyed, apparently yield different results from those with animals, in which these pathways are interrupted by hypophyseal stalk section.

Earlier reports claimed that stalk section did not inhibit the adrenal stress response to cold, trauma and histamine (e.g. CHENG *et al.* 1949; FORTIER & SELYE 1949), but this appeared to be due to the rapid regeneration of the portal vessels. HARRIS & FORTIER (1954) and FORTIER *et al.* (1957) attempted to prevent the reestablishment of the hypothalamo-hypophyseal connections by inserting a waxed-paper plate between the pituitary and the hypothalamus in rabbits. These stalk-sectioned animals showed no lymphopenia to restraint or cold, but did respond to the injection of adrenaline or laparotomy under ether anesthesia. These data are interpreted to reveal two types of stress: a neurotropic stimulus which would act exclusively via the hypothalamus, and a systemic one which would affect the pituitary directly. This interpretation is compatible with earlier data by FORTIER (1951), but the division made here seems rather artificial and does not exclude the possibility that the effectiveness of hypothalamic control is dependent on the intensity of the stressor stimulus. Nevertheless, this would implicate that the involvement of the hypothalamus is not necessarily indispensable, unless it is accepted that the quantity of released CRF would be enough to stimulate the pituitary via the systemic circulation. Similar arguments are discussed concerning the interpretation of data from experiments with animals bearing intraocular transplants of anterior pituitary tissue. Studies with pituitary glands "isolated *in vivo*" are still meeting considerable criticism, concerning the specificity of the hypophyseal response and the influence of the inevitable hypophyseal degeneration.

Data which corroborate with the dual control theory are offered by MIALHE-VOLOSS (1955), who found that the posterior lobe stores ACTH in an amount of 60—80 per cent of the anterior lobe ACTH. She reported

that a continuous noise caused an ACTH release from the posterior lobe, while histamine mainly depletes the anterior lobe ACTH (MIALHE-VOLOSS 1956). These data suggest that a neurotropic stress may liberate ACTH directly via the nervous pathways leading to the neurohypophysis, and that systemic stress acts on the anterior pituitary (MIALHE-VOLOSS 1958).

Evidence is accumulating recently that afferent impulses activating the hypothalamus during stress are mediated by the brain stem. In cats and monkeys PORTER (1953) showed that decortication and neuraxis transection up to the midbrain did not alter the stimulating effect of adrenaline on the electrical activity of the hypothalamus. Only if the midbrain is transected just behind the mamillary bodies, the adrenaline effect is abolished, SAYERS (1957) measured the blood ACTH content of stressed adrenalectomized rats, in which the brain stem was transected at the upper level of the pons, in hypophysectomized rats on the adrenal ascorbic acid depletion. He found that no elevation of the ACTH titer occurred after application of cold, trauma, ether, histamine or Pitressin as stressor stimuli (Fig. 5).

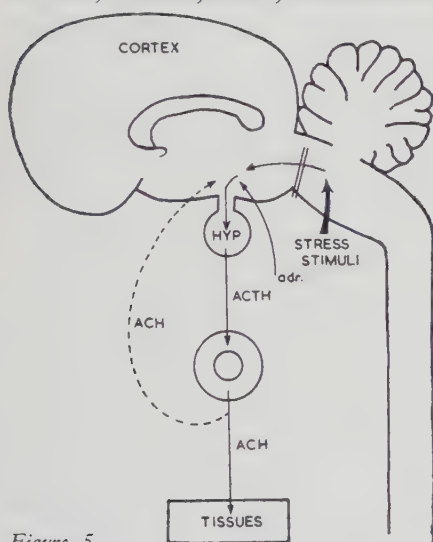


Figure 5.

Scheme representing the view of SAYERS (1957) on the role of the midbrain in the regulation of the ACTH secretion.

doubtedly established, in spite of all objections. It seems, however, that these objections are not so much directed against this fact itself as against the view, that any stressful situation invariably evokes via an unknown first mediator the release of the hypothalamic CRF into the portal circulation, which factor stimulates the anterior pituitary for a consequent ACTH depletion. This view may be oversimplified and cannot account for nu-

These interesting data are corroborated by MARTINI *et al.* (1958) for unilateral adrenalectomy and laparotomy, but not for Pitressin. ANDERSON *et al.* (1957) found in dogs that midbrain transection inhibited the increase in urinary steroid excretion following stress in normal animals. As stressful procedures laparotomy, hyperthermia, bright light for two days, restraintment and insertion of a catheter into the bladder were used. In spinal dogs laparotomy induced a striking elevation of the urinary steroids. They postulated therefore a "midbrain hypothalamico-pituitary activating system".

A direct hypothalamic control of the hypophysial function is un-

merous additional data. This will be discussed below, but the investigations on the role of the autonomic nervous system during stress and the integrative controlling function of the hypothalamus in the vegetative system will be reviewed first.

3. THE AUTONOMIC-NERVOUS CONCEPT

A. *The concept of the "irritation syndrome"*

In the same time in which SELYE made his early discoveries leading to his stress theory, an analogous concept was born in France, which is known as the "irritation syndrome" or the "phenomena of REILLY".

It was shown by REILLY and co-workers (1934, 1935) that the typhoid endotoxin produces its well-known intestinal lesions by means of the vegetative nerve fibres. When a very small amount of typhoid endotoxin, which normally does not exert any harmful effect, is placed in contact with the splanchnic nerves, a rapid development of intestinal haemorrhages and necrotic ulcers follows. They concluded that the pathogenic mechanism of typhoid fever, often observed in clinical cases, consists of a general reaction of the autonomic nervous system. Concerning the nature of the nervous mediation, it was known that normal electrical stimulation of sympathetic nerves results only in a vasoconstriction of the innervated tissues. REILLY *et al.* (1935) showed, however, that intense faradic excitation of sympathetic nerves did not produce capillary vasoconstriction, but a strong vasodilation, and by prolonged excitation even haemorrhages and necrotic areas, similar to those observed in cases of infection diseases. It appeared to be possible to reproduce the lesion phenomena by irritation of sympathetic nerves, this irritation being caused by means of faradic current, mechanical traumatizing, chemical irritants, toxic agents etc. It is not necessary for an irritating stimulus to be in close contact with sympathetic fibres; if the stimulus is strong enough, these fibres are indirectly affected and lesions will occur unless the tissues are denervated. REILLY & TOURNIER (1954) showed that injection of noxious agents into any mucosa rich in autonomic nerve fibres, produced visceral and even cerebral lesions.

Apparently this reaction is non-specific, for it responds to all types of noxious stimuli in the same way. The mediation by the autonomic nervous system explains the non-specificity of the reaction and the wide-spread occurrence of the lesions.

The pathological changes can be marshalled according to the intensity of the stimulus as follows: initially vasodilation of the capillary bed occurs,

which is accompanied by increased permeability of the capillary walls, causing exoserosis and edema. Then the capillary endothelium is ruptured, due to the extreme dilation, and the formed blood elements invade the parenchyma. This diapedesis may become so intense, that it is recognized with the naked eye as a haemorrhage. Ultimately, a necrosis of the lesioned area occurs.

These "histo-physiological" reactions are accompanied by a series of humoral reactions as changes in the blood pressure, hypoglycemia and other metabolic changes. BROCARD (1936) assumed as a result of his studies on the liver reaction to noxious substances, that irritation of the sympathetic nervous system causes all these changes by two mechanisms: 1. a direct nervous action, producing the haemorrhages, and 2. an indirect humoral mechanism, consisting of adrenal activation. The adrenal activity is enhanced by direct nervous excitation of the adrenal medulla, causing the release of sympathins, and indirectly by stimulation of the hypophysial adrenocorticotrophic principles, causing an increase in the production of corticosteroids.

The intimate mechanism of the irritation syndrome is not yet established definitely. It was suggested by TINEL *et al.* (1935) that these phenomena are caused by a local release of histamine. It is known that histamine is capable to produce vasodilation and increased permeability of the capillary bed. However, the irritation phenomena could not be antagonized by anti-histaminics (DECOURT 1954). It has also been postulated that the trophic influence of the autonomic nervous system on the cells, particularly on the reticulo-endothelial and lymphoid tissues, is destroyed by irritation, which would have serious consequences. TARDIEU & TARDIEU (1948) suggested that the irritation reactions are due to arterial vasoconstriction. Mechanical constriction of arteries by a ligature produces the same phenomena. They suppose that anoxia, and perhaps also CO₂ production, cause the capillary reactions, which start with intense vasodilation. This dilation is induced by a shortening of the endothelial cells, which normally are extended. As a consequence the intercellular spaces increase in size, causing increased permeability. If these ischemic conditions are removed by a sudden termination of the arterial spasm, the blood is permitted to invade the lesioned capillary bed and causes haemorrhages. A similar explanation is given by LABORIT (1954).

REILLY & TOURNIER (1954) claimed that a substance isolated from autonomic ganglia of animals died in irritation shock, is able to reproduce similar lesions in normal animals. They reported that it is unlikely that this substance is identical with histamine, leucotoxin or catechol amines.

The conclusions from the investigations by REILLY and his school may be summarized as follows, according to DECOURT (1951):

1. the autonomic nervous system is far more susceptible for noxious agents than other tissues;
2. the autonomic nervous system is particularly connected with the cells of the reticular-endothelial, lymphoid and endocrine systems, and these systems react mutually;
3. the organism is capable by means of these systems to respond to noxious stimuli at a distance with non-specific reactions;
4. strong and short stimuli cause vasodilation, edema, haemorrhages and necrosis; weaker and long-lasting stimuli may be antagonized successfully by compensatory mechanisms;
5. the cellular lesions occurring after the application of severe noxious stimuli are resulting from a general excitation of the sympathetic nervous system, which in turn activates the endocrine system, notably the hypophysial-adrenocortical axis and the adrenal medulla.

B. *Further research based on this concept*

Some attention will be paid now to certain aspects of the more recent investigations, which are of importance for our subject.

Originally REILLY was convinced that the efferent sympathetic nerves represent the pathway for the irritation impulses. This may be true indeed in the case of direct irritation of efferent fibres. However, already in 1935 TINEL assumed, that the mechanism would consist of an axon reflex. From other investigations it appeared that the irritation reaction is due to a centrally evoked reflex, if unspecific and systemic noxious stimuli are applied. The observations of AIDAR *et al.* (1952) show that afferent impulses from the splanchnic nerve ascend the CNS, at least as high as the thalamus. TARDIEU (1942) produced visceral lesions by injecting very small amounts of typhoid toxin into the third ventricle. He also showed the existence of pathogenic impulses passing from the brain to the periphery during the development of the phenomena of REILLY (TARDIEU *et al.* 1954). COUJARD & CHEVREAU (1955) stated that unilateral diencephalic lesions produce the same peripheral phenomena as irritation of the sympathetic nervous system. They concluded that possibly the irritation of the sympathetic nerves is realized via excitation of diencephalic areas. These experiments show that a trophic action of the hypothalamus on the peripheral autonomic neurons is present, and may account for the identity of the observed lesions. Quite recently, RICHTER (1958) suggests

that the central nervous system, particularly the diencephalon, is more susceptible for noxious stimuli than other tissues, and will be damaged in cases of extremely severe stress. Rats subjected to chronic severe stress, show permanent pathological symptoms, e.g. polyphagia, obesity, abnormal cycles in activity, food and water intake, and spontaneously recurring periods of "pseudopregnancy". At autopsy fatty livers, atrophy of the thymolymphatic tissue, stomach ulcers and pancreatic lesions were observed. These results in our opinion may be interpreted as irritation phenomena in the higher autonomic centres.

It may be concluded that reflex excitation of autonomic areas of the central nervous system might cause the phenomena of REILLY.

Pharmacological investigations are pointing in the same direction. Already since 1935 it has been tried to find drugs being capable to prevent the irritation syndrome. A number of substances were tested, among which sympathicolytic, adrenolytic, anti-histaminic, parasympathicolytic, ganglionblocking, narcotic and other drugs, given alone as well as in combination. Neither of these drugs could exert any sufficient protection (TARDIEU *et al.* 1954, DECOURT 1954). Some blocking influence has been claimed of "lytic cocktails", combinations of phenothiazine derivatives with anti-adrenergic and narcotic drugs (LABORIT 1950). The improvement of post-traumatic conditions by depression of the sympathetic activity in this way, combined with hypothermia ("artificial hibernation") led to further research with phenothiazine derivatives. This work, performed by DECOURT and others in the Rhône-Poulenc Laboratories of Specia, was awarded by the discovery of chlorpromazine (Largactil). The protecting effect of chlorpromazine was stated by REILLY *et al.* (1953), TARDIEU *et al.* (1954), and others. It was shown that the mortality rate in animals subjected to peripheral or central irritation was significantly lowered.

It is apparent from the experiments mentioned above that the protecting action of chlorpromazine cannot be due to its peripheral adrenolytic or antihistamine properties. Therefore the attention has been focused upon its central action. DELL and co-workers showed that chlorpromazine is capable to depress the electrical activity of the reticular activating system (HIEBEL *et al.* 1954). They showed that arousal reactions to sensoric stimuli are abolished by chlorpromazine. Moreover, the arousal reaction of the ascending reticular formation caused by adrenaline, is also abolished by this drug. They concluded that a powerful central adrenolytic action of chlorpromazine is added to its peripheral adrenolytic properties.

DECOURT (1953) claimed that chlorpromazine has a depressant effect on all cellular activity ("narcobiotic action"). He demonstrated this de-

pression in lower animals without nervous tissue and in unicellular organisms (DECOURT & ANGUERA 1953). This action would be compatible with the effect on the reticular formations, for he argues that this region may be particularly susceptible for the narcobiotic action, due to its reticular structure. The polysynaptic nature of this nervous network may accumulate its effect by forcing an impulse to pass through numerous nerve cells before leaving it as an effector impulse.

It has also been established that chlorpromazine inhibits the release of ACTH following stress (ARON *et al.* 1953, CASTAIGNE 1954, OLLING & DEWIED 1956, SEVY *et al.* 1957), presumably by depressing the posterior part of the hypothalamus.

The general interest in the pharmacological properties of chlorpromazine has been shifted nowadays mainly to its therapeutic value in neurotic cases, particularly those associated with autonomic disorders (the "tranquillizing" effect). Nevertheless, the earlier chlorpromazine research stresses the central position of the brain stem in the regulation of the homeostatic reactions of the organism to harmful conditions. The successful inhibition of stress phenomena by its remarkable peripheral and central anti-adrenergic action demonstrated the important role of the sympathetic system in emergency cases, as was assumed by CANNON already 30 years ago.

4. DISCUSSION OF THE REVIEWED CONCEPTS

It is often said that only facts build science. This may be true in so far that they represent the necessary building material, but it is also known that the architecture determines the usefulness of the building. It is the concept which fertilizes the scientific mind and gives the impetus to further fruitful research. However, a concept also confines the research to the framework of its own trends of thought, which means that any result of scientific work must bear the mark of subjective intuition and personal ideas. Therefore it is most desirable that different concepts get an opportunity to be confronted with each other. This opportunity has scarcely been seized in the case of the discussed concepts of SELYE and of REILLY. In our opinion this is mainly due to language difficulties. The French papers have hardly been read in the English-speaking countries. An attempt to compare the two concepts has been made by DECOURT (1951, 1952 a, b, c), but his discussions are also written in French . . .

A survey of his criticism may be summarized first. The following main points are made by DECOURT:

1. The assertion of SELYE that his notion of the "alarm reaction"

which is always the same unspecific response to any noxious agent, would be new in history, is not true.

2. The histological lesions ("gastro-intestinal ulcers") observed by SELYE are identical with the phenomena of REILLY.
3. SELYE could induce these lesions only by administration of large amounts of noxious substances, whereas REILLY was able to produce the same phenomena by placing very small amounts of the agents in contact with sympathetic nerves. Apparently the amounts given by SELYE were necessary to cause a sufficient irritation of the sympathetic nerve fibres at a distance.
4. According to SELYE the "first mediator" of the stress reaction is unknown. The mediation is shown by REILLY to be the sympathetic nervous system, being far more susceptible for noxious stimuli than other tissues.
5. SELYE explains the occurrence of the intestinal lesions by hyperactivity of the adrenal cortex, mainly through the effects of mineralocorticoids. This opinion, derived from the observation that massive doses of DOCA cause intestinal ulcers is not convincing, for small amounts of DOCA, if injected in the neighbourhood of the splanchnic nerves, produced the same lesions (DECOURT 1952 b).

Occasionally SELYE discussed the criticism made by DECOURT and others. In reply he stated repeatedly that he is not capable to cover the field of neurology. "The nervous system also plays an important role, but I did not discuss this in any detail because I have little personal experience in this field . . ." (SELYE 1952). "Several investigators expressed the opinion that in our studies on the G-A-S we failed to recognize the important part played by the nervous system, although the latter had long been known to act as a major factor regulating homeostasis and co-ordinating the various parts of the organism. It is undoubtedly true that our investigations were aimed primarily at a clarification of the hormonal factors in stress responses. For those of us who have been trained in the field of endocrinology, this seemed to be a more fruitful approach than the neurological one, for which we had no competence. In all our major publications, however, we were especially anxious to point out clearly that this one-sided approach is forced upon us merely by the limitations of our knowledge, and that the G-A-S is by no means solely an endocrine reaction" (SELYE & HORAVA 1952). One may ask here why it was not permitted to fill the gaps in his own knowledge by the results of other workers, and also if the composition of a general stress theory does not make higher demands than an experimental approach.

SELYE would even go so far that he admits: "the important investigations of Ricker, Reilly and Speranski focused our attention upon the nervous system as an important link in the pathogenesis of disease in general. Our work on the adaptation syndrome re-emphasized this point by showing that nervous irritation is an extraordinarily powerful stressor. Transection of the spinal cord, psychic stress, hypothalamic lesions, all tend to produce G-A-S manifestations qualitatively similar to those elicited by other agents, but of unusual intensity Certainly, much could be gained by a further elucidation of the manifold interrelations which exist during stress, between the two great co-ordinating systems of the body: the nervous and the humoral systems" (SELYE 1952).

SELYE's generous admission that his concept is a one-sided endocrinological approach, may clarify the situation. "Our facts must be correct. Our theories need not be, if they help us to discover important new facts" (SELYE 1952). It is apparent that, although he repeatedly asserted that he always recognized the "important role of the nervous system" (cf. also SELYE & HORAVA 1953), he never considered the possibility that not only nervous irritation is a powerful stressor stimulus, but that also within the nervous system the integrative mechanism is situated, to which the damage is informed and which consequently mobilizes the subordinated systems, e.g. the endocrine system. Even the discovery of the controlling influence of the hypothalamus did not alter the central position of the pituitary-adrenal system in his schemes (cf. Fig. 1), and still in 1956 he asserted that "yet, very little has been learned up to now about the pathways through which the hypophyseo-adrenocortical responses to stress might be connected with the nervous system" (SELYE & HEUSER 1956).

Concerning the quoted criticism by DECOURT, he may be right in several points. Undoubtedly, the views of the REILLY school are less one-sided, witness the reviewed scheme of BROCARD (1936). Nevertheless, he did not take into account the immense progress in endocrinological research and medical treatment which to a great extent were possible thanks to SELYE's unifying concept. SELYE is probably right in stating that to his mind "the most important contribution of research on stress is that it has furnished an objective, scientific basis for the development of a new approach to the treatment of disease. This is neither specific causal nor specific symptomatic therapy, but a treatment based upon the initiation and perfection of Nature's own auto-pharmacological responses" (SELYE 1952).

It is also true that the irritation syndrome reveals only one aspect of the stress reaction. But in our opinion the notion of the major role played by the autonomic nervous system appeared to be nearer at the

truth than the pleading for the hypophysis as the main organ reactive to stress. The G-A-S is not abolished by hypophysectomy or adrenalectomy, but by chlorpromazine or diencephalic lesions.

The general impression of the historical development of both concepts is that they started from different random observations on either endocrine or autonomic nervous phenomena and consequently have developed to generalized theories which were still limited by their own starting-points. It is interesting to see how the research in both fundamentally different lines came to meet each other in another field, namely the central nervous system. I tried to point out that the endocrinological stress research recently has been arrived at brain stem mechanisms, particularly the mesencephalic area, as the major site of stress control, while at the same time the school of REILLY was forced to consider the same structures as governing the entire stress mechanism.

In connection with the scope of our study, more attention will be paid now to the specific research on the integrative structure for homeostatic regulation, the hypothalamus.

5. THE ROLE OF THE HYPOTHALAMUS IN AUTONOMIC REGULATION

The experimental work on the hypothalamic activities during the last thirty years has revealed such an astonishing quantity of distinct functions, that it is impossible to review them in detail here. The earlier work done by the school of RANSON and others has been reviewed extensively by RANSON & MAGOUN (1939). Suffice it to give an impression of the autonomic effects first.

The hypothalamus exerts its influence on the periphery through direct nervous connections and through the endocrine system via the hypophysis. It is known that it controls more or less the production and secretion of the thyroid, gonadal and adrenal glands, perhaps also of the pancreas. By many of the hormones of these glands the metabolism is controlled. Further hypothalamic influences are known on: heart and pulse rate, blood pressure, respiration, body temperature, food and water intake, intestinal peristaltic movements, gastric secretion, defecation and micturition, renal reabsorption of water, secretion of salivatory and sweat glands, pupil size, muscular tone, uterine contraction, etc. In summary, the entire vegetative system is controlled by the diencephalon (cf. WEIL & BERNFELD 1955). All these phenomena and functions can be affected by electrical stimulation of the hypothalamus. This stimulation has been performed mainly by the two following techniques: faradic current under light anesthesia, the animal being placed in a stereotaxic instrument

(RANSON), or damped direct low frequency current pulses via permanently implanted electrodes without anesthesia permitting free movements of the animal (HESS).

It was recognized rather early that opposite effects could be obtained by stimulation of different regions of the hypothalamus. Thus a raise of the blood pressure and dilatation of the pupils follow from stimulation of the caudal part, while lowering of the blood pressure and pupillary constriction is caused by activation of the rostral area. It appeared that mainly sympathetic effects originate from the posterior part of the hypothalamus, whereas parasympathetic effects correspond with more rostrally and laterally situated regions.

A second feature is that one single effect can be produced by stimulation of a rather extensive area, and that it consequently is not represented in one circumscribed focus, as is the case in the cerebral cortex. Moreover, from the same point different effects can be obtained, frequently occurring simultaneously. This suggests that several neural systems are interwoven in a reticular structure.

The functional significance of these phenomena has been clarified by HESS, who started with his publications on this subject in 1931. He summarized his work, that has been awarded with the Nobel Prize, in several monographs (see HESS 1948, 1949, 1954).

His experiments showed that a localized electrical stimulation of the cat's hypothalamus does not cause only one distinct effect at large, but an organized response, composed of several effects making up a functional pattern. The exploration of the topographic distribution of these functions revealed that in the diencephalic area two autonomic tasks of the organism are represented. The first one consists of the integrative mechanism by which the organism is capable to mobilize its energies for directed action in response to external and internal stimuli like danger, appearance of sexual partner or enemies, hunger, emotions. HESS called this type of function "*ergotropic*". The ergotropic functions can be induced from stimulation of the caudal part of the hypothalamus; they are mediated in the periphery mainly through the sympathetic nervous system, but also partly through somatic effectors (e.g. in enhanced respiration and locomotion) or parasympathetic nerves. Common autonomic phenomena of ergotropic stimulation are: raise of blood pressure, increase in pulse rate, pupillary dilatation, increased respiration, affective behaviour.

The second task to be performed by the hypothalamus is the protection, restitution and saving of the energies. This concerns the mechanisms of rest, anabolic processes and repression of energy release, and thus represents the counterpart of the ergotropic functions. It is called the

trophotropic system, and it is mainly mediated by the parasympathetic nervous system. Its activation causes e.g. slowing of the heart rate, lowering of the blood pressure, micturition, defecation, salivation, chewing, vomiting and sleep.

Thus the well-known peripheral effects of sympathetic and parasympathetic activity are integrated and controlled by the hypothalamus. Either activity is represented in a different area of the hypothalamus, although these areas are not clearly limited and overlap each other partly.

It has been said already that stimulation evokes a series of ergotropic or trophotropic effects, appearing simultaneously and integrated in a purposeful function. The methods employed by HESS enabled him to observe these functions in freely moving and normally behaving cats. Emotional behaviour is elicited from the ergotropic area, particularly from the so-called "dynamogenous zone", which induces regular attacks or flight. These "affective defense reactions" are the expression of true emotion, differing from the well-known "sham rage". BARD (1928) showed that decortication causes signs of anger, but the phenomena of spontaneous undirected effects in decorticated animals were rightly called "pseudo-emotional". Direct stimulation in intact animals creates a state of true emotion: the animal attacks an object, e.g. the nearest human being. For this behavioural response a high integration of autonomic functions, sensoric stimuli and motor activity is necessary, which involves the cooperation with cortical information. Apparently an activation of certain hypothalamic structures may cause not only downward, but also upward discharges, activating cortical areas.

In another area, just caudal to that for rage or flight a different behaviour can be elicited, namely bulimia. The animal will search around for food, and eat everything, even inedible objects, with great voracity. Still more posteriorly, a motor behaviour can be induced which urges the animal to move eagerly around without any special goal. This restless behaviour may be identical to the "appetitive behaviour" in the ethological terminology. DELL (1958) argues that the state of alertness and motor behaviour is created by activation of the reticular formation. Examples of complete behavioural acts are also known from the trophotropic region. Stimulation of the "hypnogenous zone" produces true sleep and searching for a comfortable posture. Also defecation can be evoked which occurs in the adequate position.

These examples show that instinctive behaviour, consisting of genetically fixed motor patterns can be elicited by electrical excitation of the hypothalamus. This implies that the diencephalon has important integrative functions. In order to perform these acts the hypothalamus

has to control the main peripheral systems. This does not mean, however, that the diencephalon would sit alone at the head of the autonomic table. It must be regarded as part of many complex neural circuits involving certain cortical and brain stem areas. It has connections with the prefrontal lobes, the gyrus cinguli, the rhinencephalon (limbic system) and higher brain stem systems as the ascending reticular formation, these connections being afferent as well as efferent. Most of these circuits are poorly understood and the anatomical evidence is often scarce, but various physiological experiments show that they must exist. There is some evidence for an "emotional circuit" (PAPEZ 1937), which may be activated in the reviewed experiments of HESS. The affective defense reaction has been caused by stimulation of the mamillary area and the perifornical zone. This is very well in accordance with the idea that the limbic system (hippocampal and amygdaloid structures) is involved in emotional behaviour (GREEN 1956, GLOOR 1956). This system projects on the hypothalamus through the fornix, running from the hippocampus towards the mamillary bodies and the stria terminalis, terminating in the tuberal part of the hypothalamus. Upward discharges from the mamillary bodies run via the mamillo-thalamic tract. In the case of HESS's experiment the emotional behaviour is not initiated by external stimuli, as normally will occur, but once in action it is guided by these stimuli, acting as feed-back mechanisms. This means that the hypothalamus in turn activates the neocortex, which must assimilate the sensoric information, in order to inform and activate the palaeocortex (limbic system). This example shows that the hypothalamus is imbedded in an interplay of forces, that it controls a complex pattern of autonomic and somatic functions, but also is regulated in turn by cortical impulses through neural circuits.

Also the interrelation of the two autonomic systems, the ergotropic and the trophotropic mechanisms, does not show a fixed and invariable pattern, but depends on the state of responsibility of the concerned areas, and is a matter of balancing interplay, influenced by lower and higher structures. This has been the subject of much experimental work done by GELLHORN, who summarized his views in a monograph (GELLHORN 1957).

In cats he injected hypotensive or hypertensive drugs and used the blood pressure, pulse rate and nictitating membrane as indicators for autonomic activity. He showed that a change in the blood pressure induces a compensatory shift of the autonomic balance, which opposes the original effect. Thus, a hypotensive drug causes a fall of the blood pressure, and consequently the autonomic balance is shifted to the sympathetic side, which can be seen from an enhanced contraction of the

nictitating membrane and a compensatory increase in blood pressure and heart rate. This phenomenon is mediated by a sino-aortic baroreceptor reflex, for after sino-aortic denervation it is abolished. The shift is called "sympathetic" or "parasympathetic tuning", and is thought to originate from a change in hypothalamic balance between the sympathetic (ergotropic) and parasympathetic (trophotropic) side. The state of autonomic tuning can be demonstrated by direct stimulation of the hypothalamus. During sympathetic tuning the effect of excitation of the posterior (sympathetic) hypothalamic area is enhanced, during parasympathetic tuning it is decreased. The effect of this direct stimulation is increased even if the amount of previously injected hypotensive drug was too small to evoke an observable sympathetic reaction of the nictitating membrane, which indicates that the hypothalamus is very sensitive for these reflex stimuli. Not only the sympathetic reactivity of the posterior hypothalamus is increased during sympathetic tuning, but the parasympathetic reactivity of the anterior part is diminished as well. A change in the autonomic balance apparently causes an increase of the responsibility of the one system with a simultaneous decrease of the sensitivity of the antagonistic system, which argues for the existence of reciprocal innervation.

In the cat normally the sympathetic division is dominating and obscures the parasympathetic action. If noradrenaline is injected causing the condition of parasympathetic tuning, a stimulus which normally acts sympathetically (e.g. a nociceptive stimulus like immersing the tail in hot water, or direct stimulation of the posterior hypothalamus) now produces parasympathetic effects (phenomenon of reversal). This suggests that in fact these stimuli cause an excitation of both divisions of the autonomic system. It depends on the state of autonomic balance whether mainly sympathetic or parasympathetic peripheral effects are produced. A direct simultaneous and equal stimulation of the anterior and the posterior hypothalamus produces in the normal animal also a sympathetic discharge. Thus an indirect activation of the hypothalamus causing sympathetic effects does not imply that the parasympathetic division is not activated at all.

The excitability of either system is increased if the antagonistic area is inhibited directly, by hypothalamic lesions or intrahypothalamic injection of pentothal. Moreover, the tonic effects of the inhibited system appears to be abolished as well. Coagulation of the posterior hypothalamus causes a lasting decrease of the blood pressure, while the hypotensive effect of acetylcholine is increased.

With one denervated nictitating membrane serving as an indicator for the release of endogenous adrenaline it has been demonstrated, that on

mild stimulation of the posterior hypothalamus only neurogenic sympathetic discharges occur, but that stronger stimulation activates the adrenal medulla as well. GELLHORN showed that during a neurogenic discharge the reactivity of the sympathetic system is enhanced, but that during the subsequent adrenomedullary hormone discharge the responsiveness is greatly decreased, as measured on the pupillary dilatation, which is inhibited during the adrenaline secretion, causing a contraction of the denervated nictitating membrane. Asphyxia causes a strong adrenaline discharge, and during this phase the excitability of the posterior hypothalamus is diminished while the responsiveness of the anterior part is increased. GELLHORN concluded that the adrenomedullary hormones produce a parasympathetic tuning of the hypothalamus; in other words: adrenaline inhibits the sympathetic part. This is interpreted as a contribution to homeostasis (negative feed-back). This humoral feed-back mechanism comes into action during strong excitation of the sympathetic system.

This view is a correction of CANNON's theory which supposed a synergistic action of neurogenic and hormonal sympathetic factors. It is not in accordance with the findings of PORTER (1953) in anesthetized cats who claimed that adrenaline stimulates the posterior hypothalamus. Perhaps the controversion is mainly due to the different use of anesthetics. GELLHORN normally used a combined chloralose and urethane anesthesia, and measured the effect of endogenous adrenaline in sympathetically activated animals, whereas PORTER injected adrenaline in cats anesthetized with cyclopropane. Moreover, the interpretation of encephalo-electrograms under anesthesia is doubtful and cannot be simply applied to conscious animals. From teleological reasoning the idea that adrenaline inhibits the activity of the caudal hypothalamus is more obvious, as an enhanced activity of this region is known to cause a release of adrenaline from the adrenal medulla, and adrenaline would then act as a positive feed-back mechanism.

As has been reviewed already, HESS showed that stimulation of the posterior hypothalamus may induce directed emotional behaviour, which demonstrates that the cerebral cortex is involved in this reaction. GELLHORN also claims to have demonstrated influences of the posterior hypothalamus on the cortex and *vice versa*. He argues that the caudal hypothalamus is part of the reticular-thalamic-cortical system of MAGOUN. Stimulation of the posterior hypothalamus exerts an excitatory effect on the cortex. Alterations of the autonomic balance at the hypothalamic level alter this hypothalamic-cortical discharge. Direct excitation of the cortex evokes an increase in the rate of hypothalamic discharges and of the responsiveness

to various stimuli. Since conditions leading to a state of increased excitability of the hypothalamus induce "upward" discharges, corticofugal discharges apparently can induce also corticopetal discharges. In this feed-back circuit the posterior hypothalamus (and the reticular formation) have a key position. These facts may provide according to GELLHORN a physiological understanding of emotional behaviour and psychotherapy. They indicate that cortical processes may cause changes in emotional reactivity, but that they also can be altered themselves in various emotional states.

FUNKENSTEIN *et al.* (1952) noted that changes in the blood pressure reaction to mecholyl, a hypotensive drug, were associated with changes in the psychological behaviour of patients. It was shown by GELLHORN that mecholyl causes an excitation of the posterior hypothalamus, which leads to a downward and an upward discharge. Therefore, a change in the mecholyl test reflects also a change in the corticopetal discharge, which is intimately related to perception and behaviour. FUNKENSTEIN (1955) suggested that persons being hyperreactive in the mecholyl test, were "angry at others", while hyporeactors were "angry at themselves". He argued that rage, as in the hyperreactors, causes a secretion of noradrenaline while fear, as in the hyporeactors, would be associated with an adrenaline discharge. These data are corroborated by the work of ELMADJIAN *et al.* (1958). It is interesting to compare these assumptions with some data from the work of HESS, who noted that during continued stimulation of the dynamogenous zone a change in emotional behaviour may occur from rage to fear, i.e. from fight to flight, and with data offered by FOLKOW & VON EULER (1954) showing that the release of medullary adrenaline or noradrenaline on stimulation of the posterior hypothalamus is dependent on the site of stimulation. It seems reasonable to suppose that the degree of sympathetic activity of the caudal hypothalamus may induce these behavioural changes. GELLHORN suggests that the degree of hypothalamic excitability determines the ratio of adreno-medullary secretion. In anger and rage this excitability would be greater, causing an intense neurogenic discharge with a high level of peripheral noradrenaline release, while in states of fear and anxiety the hypothalamic excitability is lower, which induces to his experience an increase of adreno-medullary hormone discharge, being the chief source of adrenaline. In clinical tests GELLHORN found that psychopathic persons were mainly hyperreactive, while hyporeactors were absent among psychopaths and normals but present among schizophrenics. In both normal and neuropsychiatric human the sympathetic reactivity decreases with increasing age.

These experiments show the importance of the hypothalamic balance

in emotional behaviour and disorders. The work of HESS and GELLHORN enables us to gain more insight in the physiological significance of the hypothalamus in homeostatic regulation and adaptation to nociceptive stimuli.

Very little work has been done on the relationship between cortical processes and the ACTH secretion. Some interesting data on the influence of direct cortical stimulation on the eosinophil count in cats and monkeys are offered by PORTER (1953, 1954). The cortex is not required for the production of stress-induced eosinopenia, as an eosinopenic response to adrenaline and trauma also occur in decorticated animals. Electrical stimulation of the prefrontal cortex, particularly of the orbital surface, induces a significant eosinopenia. Stimulation of the hippocampal region reduced the eosinopenic response to a simultaneously applied stress for more than 50%; stimulation of the uncus even for 80%. These rhinencephalic regions are known to project to the hypothalamus, being part of the so-called "limbic system". An inhibitive influence of the hippocampus on the posterior hypothalamus through the fornix has also been assumed by GREEN & ARDUINI (1954), for transection of the fornix induces alert and active behaviour. This reminds one to the fact that the cortex exerts an inhibiting influence on the emotional behaviour, witness the occurrence of "sham rage" in decorticated animals. Consequently, activation of the emotional circuit might be associated with or even caused by an inhibition of the hippocampal-hypothalamic impulses.

On the other hand, MASON (1956, 1958) reported that avoidance behaviour and electrical stimulation of the infundibular region and the amygdaloid nucleus induced a raise in the level of plasma 17-hydroxycorticosteroids. The amygdala projects to the ventral hypothalamus through the stria terminalis. According to GLOOR (1956) stimulation of the amygdala causes an activation of the whole hypothalamus. Ablation of the amygdala resulted in his experience not in an increase, but in a decrease of fear and rage responses. Hippocampal lesions were quite ineffective in this respect. In his opinion the limbic system is not in actual command of the hypothalamic mechanisms, but it exerts subtle modulatory influences on them.

It is possible that excitatory and inhibitory influences on the adrenocorticotrophic function of the hypophysis both can be elicited from the limbic structures, and that in the normal animals a complicated interplay between these mechanisms modifies the activity of the hypothalamic-hypophyseal system.

6. EVALUATION OF THE REVIEWED LITERATURE AND STATEMENT OF OUR PROBLEM

The endocrinological research on the stress mechanism has mainly focused its attention upon the regulation of the ACTH secretion. The work done on the role of the adrenocortical hormones (SAYERS) and the adrenomedullary hormones (LONG) in the control of ACTH discharge pointed out that the hypothalamus is involved in this mechanism. Further work on the influence of the hypothalamus on the hypophysial functions, inspired on the neurohumoral concept of HARRIS, demonstrated that a transmitter substance must exist, which is carried through the portal vessels to the anterior lobe of the hypophysis. The existence of a hypothalamic "hormone" does not imply that the hypothalamus possesses the properties of an endocrine gland. The functional significance of the humoral transmission is still obscure, as nervous connections with the anterior lobe are also present. In which ways the hypothalamus may control the production and secretion of ACTH under normal and emergency conditions, can be best approached in our opinion when we consider the functional role of this structure in the organism. Therefore some pertinent experimental work has been reviewed, which showed that the hypothalamus holds a central place in the regulation of autonomic functions. It is divided into two areas, an ergotropic and a trophotropic region, governing the peripheral sympathetic and parasympathetic nervous system.

The ergotropic area appeared to be involved in emotional reactions, and is closely connected with the ascending reticular formation, the palaeocortex (limbic system) and the neocortex. As many forms of stress are associated with strong emotional stimuli, an activation of this neural system is obvious. The French work, done by the school of REILLY, points in the same direction. Noxious stimuli appeared to irritate the whole sympathetic division, causing vasodilation, increased capillary permeability and even diapedesis and haemorrhages in the innervated organs. The inhibiting capacity on the stress reactions (shock phase) of chlorpromazine, which drug has a strong central and a peripheral anti-adrenergic action, suggests again that these pathogenic impulses are induced by a central reflex of brain stem structures. The discharge of ACTH is one of the numerous mechanisms brought into action during ergotropic activity.

On the other hand, if the organism is exposed to a stress without emotional content, or if the emotional component is eliminated, e.g. by anesthesia, the defense mechanism still is known to be activated. In other words, there must exist more mechanisms inducing hypophysial

activation. It is possible that also a causal relation exists between activation of the trophotropic zone and the ACTH secretion. It is known that the neurosecretory material, which is supposed to be closely related to the humoral transmitter (CRF), originates from the anterior part of the hypothalamus.

In our general introduction we stated that it was our aim to study the relation between the autonomic nervous system and the ACTH secretion during stress.

From the literature we conclude that possibly the following autonomic mechanisms may stimulate the ACTH discharge:

- a.* a central influence from the diencephalon, as a result of activation of the ergotropic or/and trophotropic area, or changes in their balance;
- b.* a peripheral influence from the sympathetic nerves innervating the glandular tissue of the adenohypophysis, which may be forced by the irritation reaction to release its hormonal content.

Consequently our experimental work is concerned with the two following problems:

1. Does irritation of the sympathetic innervation of the anterior pituitary induce the phenomena of REILLY? If so, is this vasomotor mechanism capable to cause or to enhance the secretion of ACTH?
2. Does a specific influence exist of the two autonomic divisions of the hypothalamus on the ACTH liberation? If so, what is their physiological significance in stress reactions?

II

EXPERIMENTS ON THE INFLUENCE OF THE PERIPHERAL SYMPATHETIC INNERVATION ON THE STRUCTURE AND ACTIVITY OF THE PITUITARY GLAND.

1. INTRODUCTION.

The innervation of the anterior lobe of the pituitary gland is a vexed question. If a nerve supply exists, it may be received from three sources: sympathetic fibres from the cervical sympathetic chain, running with the internal carotid artery; parasympathetic fibres from the cranial nerves III, VII and IX; and central fibres coming down from the hypothalamus via the hypophyseal stalk. HARRIS (1955), reviewing the available data on this subject, concludes that very likely "the pars distalis receives few, if any, sympathetic fibres", that the parasympathetic supply does not play any part in regulating the glandular activity, and that "the pars distalis receives very few, if any, nerve fibres" originating from the hypothalamus. These conclusions are based upon the fact, that most authors were not able to observe nerve fibres within the glandular tissue (e.g. WINGSTRAND 1951, GREEN 1951 a, b). Moreover, transection of the sympathetic or parasympathetic afferent nerves had little or no effect on the physiological activity of the hypophysis.

Nevertheless, a single clear demonstration of the existence of nervous formations in the adenohypophysis is of more value than numerous negative results. Opposed to many negative reports stand some investigations which clearly indicate that a rich nervous network exists in the glandular part of the hypophysis. HAGEN (1950) described an extensive sympathetic innervation in the human hypophysis, which she thinks to be either secretory or receptory. Similar findings she reported in dogs and monkeys (HAGEN 1954). VASQUES LOPEZ (1949, 1952) in the rabbit and METUZALS (1954) in the horse found a nervous end-plexus innervating the glandular cells and originating from the infundibular region. METUZALS (1955) observed a similar end-plexus in the duck, and in addition, an autonomic nervous formation in all parts of the pituitary gland. This autonomic plexus is composed of large strands of nerve fibres, with ganglion cells mainly situated in the capsule, and of delicate fibres, belonging to the autonomic end-formation, the sympathetic groundplexus of BOEKE. This

ground-plexus continues into the glandular tissue, between the gland cell rows and the sinusoids. A similar ground-plexus appears to exist in most endocrine glands (STÖHR 1954). URAZOV (1955) found in a series of animals a sympathetic innervation, entering the hypophysis with the superior and inferior hypophysial arteries, and terminating on the capillaries and glandular cells of all parts of the adenohypophysis. He assumes that these nerves contain secretory and vasomotor fibres.

If it is taken for granted that autonomic nervous formations in the pars distalis exist, the question arises from which sources they originate. Generally the sympathetic fibres run from the superior cervical ganglion rostrally with the internal carotid artery and its branches as the deep petrosal nerve, in higher mammals as a plexus in the arterial wall; they are supposed to be mainly post-ganglionic, although smaller sympathetic ganglia and scattered ganglion cells are found also more distally from the cervical ganglion. The parasympathetic nerve supply of the hypophysis is derived mainly from fibres running with the facial nerve and branching off as the major superficial petrosal nerve. This branche joins the sympathetic carotid nerve near the pituitary, thus forming the vidian nerve which run to the sphenopalatine ganglion. At this junction ZACHARIAS (1941) discovered a small ganglion in the rat, which he called the vidian ganglion. From this ganglion fine branches are distributed directly to the capsule of the pituitary gland. The anatomical arrangement renders it likely that these strands contain sympathetic as well as parasympathetic fibres.

A comparable situation is found in the duck, in which animal we closely examined the autonomic pathways histologically and under the dissecting microscope (SMELIK & METUZALS, unpublished observations). The

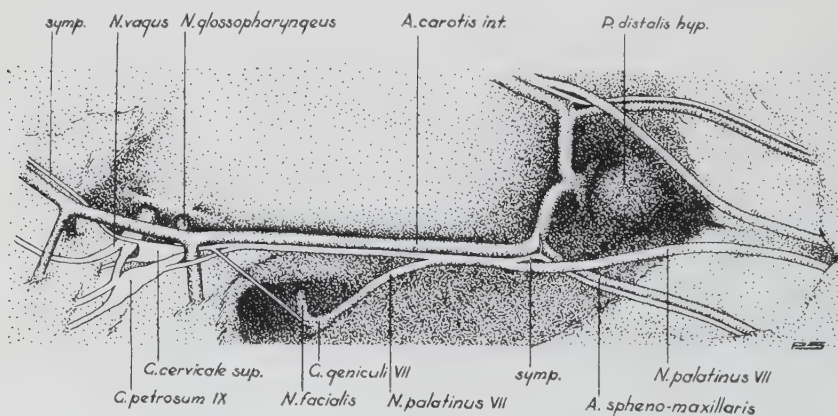


Figure 6.

Origin of autonomic innervation of the pituitary gland in the duck. The floor of the brain and the pituitary gland are exposed from the ventro-lateral side. x 20.

sympathetic nerve (cf. Fig. 6), running rostrad with the internal carotid artery, meets the palatine nerve, a branche from the facial nerve containing parasympathetic fibres. They join for a short distance, and then the sphenomaxillar branch is emitted, while the palatine nerve (= vidian nerve)

runs further to the sphenopalatine ganglion. In that part where the sympathetic and the parasympathetic nerves are fused, ganglion cells occur abundantly, their number being estimated on 2000 cells. Apparently in this intimate connection the sympathetic and the parasympathetic fibres are switched over and likely also mixed. This area may be homologous with the vidian ganglion in mammals (cf. fig. 7). Immediately after this anastomosis two small branches follow the internal carotid artery on either side towards the hypophysis. Near the capsule of the hypophysis several autonomic ganglia were observed (METUZALS 1956). Further it was noted that the sympathetic nerve receives a branche from the petrosal ganglion, which lies ventrally to the superior cervical ganglion and receives parasympathetic fibres from the vagus

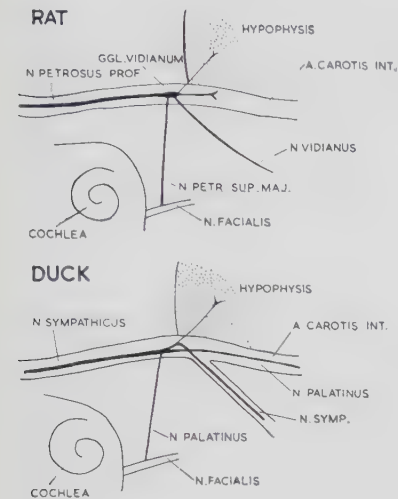


Figure 7.

Comparative schematic representation of the autonomic innervation of the pituitary gland in the rat (after ZACHARIAS 1941) and in the duck.

nerve. Probably the sympathetic nerve receives here already parasympathetic fibres. According to HALLER VON HALLERSTEIN (1939, p. 742) also no pure sympathetic cranial nerves exist in mammals. These observations show that in avian and mammalian organisms the hypophysis receives mixed autonomic nerve fibres with many intercalated ganglion cells.

Physiological experiments indicating that the autonomic nerve supply of the hypophysis plays a role in the activity of the gland are scarce. ZACHARIAS (1942) reported that bilateral extirpation of the vidian ganglion in the rat is followed by pseudopregnancy and increased insulin sensitivity; transection of the parasympathetic greater superficial petrosal nerve also caused pseudopregnancy. VOGT (1942) claimed that bilateral transection of the greater superficial petrosal nerves did not inhibit the reflex release of gonadotrophin following coitus in the rabbit. It is likely, however, that this operation did not eliminate the parasympathetic innervation of the hypophysis, since the sympathetic nerves are probably mixed with parasympathetic fibres. Moreover, pseudopregnancy and ovulation will

readily occur in unspecific interferences. ABRAMS *et al.* (1955) found that bilateral extirpation of the superior cervical ganglion inhibits the onset of oestrus in ferrets which occurs during artificial illumination in winter. However, DONOVAN & VAN DER WERFF TEN BOSCH (1956) assumed that this delay is due to the occurrence of HORNER's syndrome (pupillary constriction, enophthalmos, ptosis) causing a reduction in the quantity of light stimulating the brain stem.

After extirpation of the superior cervical ganglion COLLIN & HENNEQUIN (1936 a, b) observed capillary vasodilation and disappearance of the acidophils in the anterior lobe after three days. One week after the operation the whole picture has become chromophobic. These changes are not irreversible; they assume that the occurrence of carotid or parenchymatous microganglia is responsible for the recovery of the sympathetic tone. It may be mentioned here that neuro-anatomists are convinced that it is impossible to eliminate the autonomic nerve supply of an organ by transection of the afferent nerves. The occurrence of peripheral ganglia and nerve cells within the organ ("interstitial cells") prevents an irreversible degeneration of the autonomic plexus (cf. STÖHR 1941). The possible physiological role of the autonomic innervation cannot be demonstrated by transection of afferent pathways. Stimulation of the autonomic afferents to the hypophysis in physiological experiments has never been done. Histological changes following stimulation may be explained by vaso-motor reactions (COLLIN 1937).

Little is known of changes due to sympathetic irritation. Only some data are available from irritation of the terminal sympathetic in the gonads, which induced degenerative changes and haemorrhages in the anterior lobe, the occurrence of pycnotic nuclei, disappearance of basophil cells, and a chromophobic appearance of the central part of the glandular parenchyma, especially of the acidophil cells (CHEVREAU & COUJARD 1954).

The demonstration of irritation phenomena in the hypophysis may give some information on the existence, extent and possible significance of the autonomic nerve supply. Some pertinent experiments are described below.

II. EXPERIMENTAL WORK

A. Production of the phenomena of REILLY in the pituitary gland

Material and methods

Female albino rats of an inbred strain (*Brofo*) were used, weighing from 120—200 gm. Anesthesia was performed 10 minutes previously by an intravenous injection of 3.25 mg. Nembutal per 100 gm. body weight. A midline incision was made in the neck and the left carotid

artery was exposed. Then the sympathetic nerve, running closely to the arterial wall, was carefully separated and lifted up with two small hook-shaped electrodes. Special attention was paid not to damage the nerve mechanically. A faradic current was produced by means of a RUHMKORFF coil. Pupillary dilation was used as an indicator for the effectiveness of the stimulation. In sham-treated animals only the carotid artery was exposed, but not touched. After the faradic irritation the animal was decapitated, and the hypophysis was removed and fixed as fast as possible. The gland was fixed in SUSa's fluid, embedded in paraffin and sectioned at $5\ \mu$. The sections were stained with HEIDENHAIN's azan method or an azan modification by FARKAS (1940).

Results

1. First the effect was observed of faradic irritation of equal intensity during periods of 1, 5, 10 and 30 seconds. Decapitation followed after 12 minutes. In rats having received faradic current during 1 second a vasodilation of the hypophysial sinusoids was observed. Faradization during 5 secs. induced vasodilation and edema. Also many erythrocytes are found outside of the sinusoids between the glandular cells (*Plate I*, fig. 2). A 10-seconds irritation caused a similar extravasation of erythrocytes, but many basophil cells have also disappeared. Faradization of 30 secs. duration induced intense parenchymatous haemorrhage, while no basophil cells are discernable and the whole tissue has a chromophobic appearance. Numerous nuclei appear to be pycnotic (*Plate I*, fig. 3). In all cases the reaction was most intense in the central part of the anterior lobe. In some instances only the left half of the anterior lobe was damaged but usually the whole lobe was affected.

It was decided to use the extravasation of erythrocytes as a criterion for the occurrence of irritation reactions in further experiments.

2. The time interval, necessary for the development of the REILLY phenomena, was measured as follows. The animals were decapitated 2, 5, 15 seconds, 1, 4, 7, 10, 20 and 30 minutes after faradization of 5 secs. duration. In all cases the reaction was present. As the time interval between decapitation and fixation amounted to approximately one minute, it was attempted to reduce this interval by fixation *in vivo*. An amount of 5 ml formalin 4% was injected intracardially exactly at the end of the 5 secs. of faradization. In these animals the irritation phenomena occurred as well.

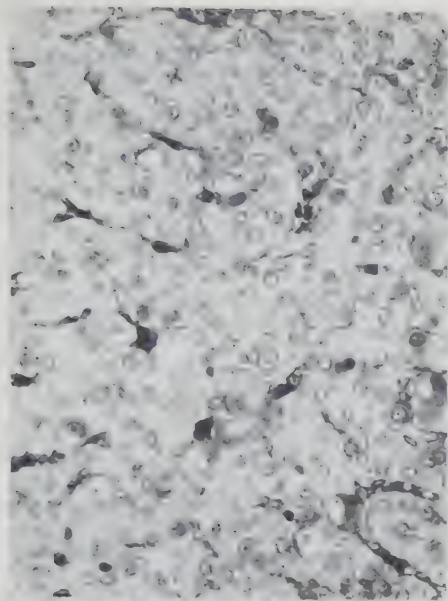
It is concluded that the reaction has been developed within 5 seconds after sympathetic irritation.

3. Attempts were made to investigate whether indirect irritation of

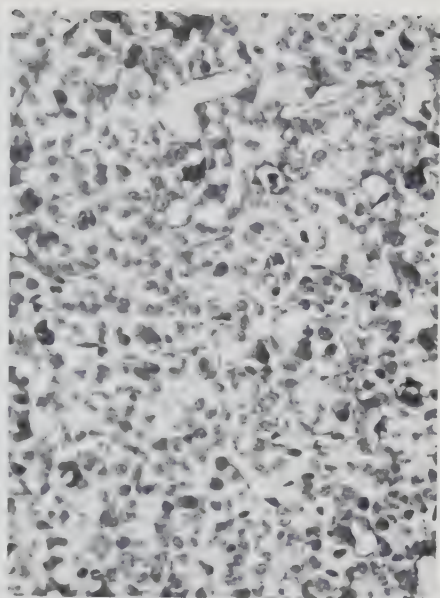
PLATE I

- Fig. 1. Normal histology of the pituitary gland (pars distalis) of the rat (Azan, x 235).
- Fig. 2. Vasodilation and edema in the pars distalis, resulting from short faradic irritation of the cervical sympathetic nerve (Azan, x 235).

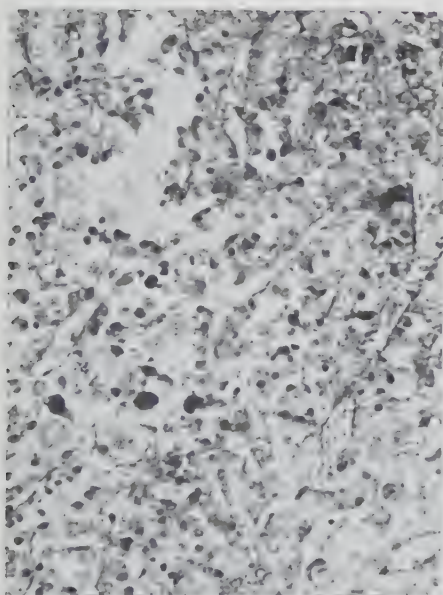
- Fig. 3. Parenchymatous haemorrhages and tissue destruction caused by prolonged sympathetic irritation (Azan, x 165).
- Fig. 4. Extensive damage of the glandular tissue as a consequence of severe cold stress during 3 hours (Azan, x 43).



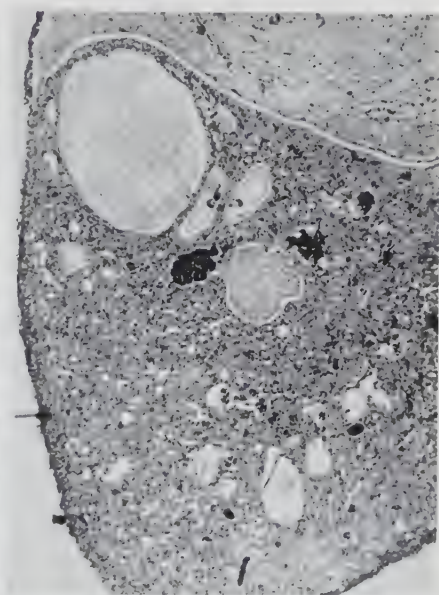
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2



3



4

the sympathetic nervous system is capable to induce the same hypophyseal reactions. Adrenaline, histamine and Pitressin were injected intravenously in order to check their possible capacity to produce sympathetic irritation. The animals were decapitated in these experiments after 30 and 60 seconds. The injection of 10—50 γ adrenaline did not cause any histological change of the anterior lobe tissue. Administration of 0.1—0.5 I.U. Pitressin caused a marked vasodilation. The same effect was produced by injection of 0.4—1.0 mg. histamine.

Neither of these drugs caused the irritation phenomena in doses generally known to be sufficient to cause an intense secretion of ACTH.

In some animals it was tried to produce the irritation reactions by unspecific stresses. From the control animals it was already known that exposure of the carotid artery under anesthesia never produced these reactions. The subcutaneous injection of 0.5 ml formalin 4% caused death in some rats within a few hours; nevertheless in none of the formalin-treated animals the hypophyseal tissue showed irritation reactions, but only strong dilatation of the sinusoids. Exposure to cold (0° C) for more than 18 hours was also ineffective, but severe cold (—15° C) for 3 hours induced a remarkable damage of the anterior lobe. (*Plate I*, fig. 4).

B. Influence of sympathetic irritation on the ACTH secretion

Material and methods

Female rats of an inbred strain (*Unilever*), weighing 135—165 gm, were used. One day prior to the experiment 5 mg Prednisolone (MERCK, SHARP & DOHME) was given subcutaneously, in order to prevent the reflex ACTH secretion to unspecific stress. In our experience this amount of Prednisolone inhibits the hypophyseal stress response without interfering with the hypophyseal reactivity to direct stimulation by Pitressin (SMELIK & DEWIED 1958).

The animals were anesthetized with 3.25 mg Nembutal per 100 gm. body weight, injected intravenously 15 minutes before the actual experiment. In all animals the carotid artery was exposed. The cervical sympathetic nerve was faradized during 15 secs. in the experimental group; non-faradized animals served as a control group. After one hour the animals were sacrificed by decapitation, and both adrenals were removed. The adrenal ascorbic acid content was measured according to the method of ROE & KÜTHER (1943). The pituitary glands were removed for histological examination. For statistical methods see p. 58.

Results

Table 1 shows that the exposure to faradic irritation caused a significant

ACTH secretion in two experiments. The mean ascorbic acid depletion amounted to 63 mg%. In all but one faradized animal the histological appearance of the anterior lobe of the pituitary showed irritation reactions. The non-effective faradized rat was discarded.

TABLE 1

The effect of faradic irritation of the cervical sympathetic nerve on the ACTH release from the hypophysis in Prednisolone-pretreated rats.

Experiment	Adrenal Ascorbic Acid Content ¹⁾ 1 hour after		Double tail probability
	Sham-operation	Faradic irritation	
I	508 ± 11.3 (10)	446 ± 23.5 (9)	p = 0.05
II	488 ± 7.9 (10)	425 ± 14.5 (10)	p < 0.01
I + II	498 ± 6.9 (20)	435 ± 14.1 (19)	p < 0.01

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

III. DISCUSSION

These data show that a sympathetic innervation of the pituitary anterior lobe exists, and that its irritation causes serious damage to the glandular tissue. The irritation phenomena could be reproduced in the same order of intensity as was found by REILLY (cf. p. 24). Our results indicate that the tissular reaction responds very fast to the irritation, at most within 5 seconds.

It is difficult to evaluate the possible significance of this phenomenon. Well-known stressor agents like histamine, Pitressin and formalin only cause strong vasodilation. This is also true for a trauma like exposure of the carotid artery. Vasodilation was not found after injection of adrenaline, but as the animals were decapitated immediately afterwards, vasodilation might have occurred after a longer time interval. The data are not in favour of the view, that the irritation phenomenon is due to locally released histamine. Similar data are reported by ROTHBALER (1953, 1956), who found a marked vasodilation within 2 minutes after painful and emotional stimuli. Thus an activation of the hypophysis is associated with vasodilation.

This dilatation will cause an increased permeability of the capillary walls. Such a mechanism may be indispensable for the discharge of the hormonal content of the glandular cells into the sinusoids. An activating stimulus enhancing the secretory functions of the gland presupposes the cooperation

of releasing mechanisms. For the intimate regulation of the tissular reactivity and vasomotor changes the autonomic innervation is certainly of great importance. A secretory influence of autonomic nerve impulses is not observed in our experiments.

When as a result of faradic irritation, the capillary walls are disrupted, the glandular parenchyma comes in direct contact with the blood and the colloid can be picked up by the flowing bloodstream. In this way a hormonal discharge might occur. This is not substantiated in our experiment, in which the ACTH output is measured after short faradization in Prednisolone-inhibited animals. The ACTH discharge is slight, and we assume, therefore, that a direct contact between the secretory cells and the blood stream is not sufficient for a liberation of ACTH. A central stimulus (CRF?) must reach the gland cells as well, in order to activate the secretory functions. The release or the effect of this central stimulus has been blocked in our experiment by a high blood level of corticosteroids, whereas the irritation phenomena were not inhibited.

It is possible that in lower organisms the REILLY phenomena occur also under physiological conditions, since METUZALS (personal communication) observed an extravasation of erythrocytes in the hypophysis of fishes during a period of high endocrine activity. In mammalian organisms these phenomena are likely to occur only during extremely severe stress. Moderate cold induces merely vasodilation of the sinusoids, indicating an enhanced activity of the pituitary (cf. BAILLIF 1938), but intense cold appeared to induce degenerative changes in the glandular tissue, which may occur in a stage of exhaustion. In this connection it may be recalled to mind that similar hypophysial injuries are known from several cases of SIMMOND's disease. For instance the pathological changes described in the syndrome of SHEEHAN are supposed to be due to thrombosis of the hypophysial vessels, but similar vasomotor troubles are caused by sympathetic irritation according to TARDIEU (cf. p. 25). It is significant that this syndrome appears after severe stress, i.e. difficult labour accompanied by haemorrhage or toxæmia.

We conclude that it is possible to reproduce the phenomena of REILLY in hypophysial tissue by irritation of the sympathetic innervation. This irritation may induce a slight discharge of ACTH (and likely of other hypophysial hormones) by means of parenchymatous haemorrhages. In mammals this reaction does not occur under physiological conditions and mild stress, but extremely severe chronic stress is able to induce irritation phenomena in the pituitary gland. We did not find an argument for a role of the autonomic innervation other than vasomotor.

III

EXPERIMENTS ON THE INFLUENCE OF HYPOTHALAMIC STRUCTURES ON THE STRESS-INDUCED ACTH SECRETION

1. INTRODUCTION

The experiments described in this chapter are mainly done with the aid of hypothalamic lesions. It was our aim to investigate the effect of lesions destroying the anterior (trophotropic), tuberal and posterior (ergotropic) region of the hypothalamus on the ACTH secretion following acute stress.

The results of the numerous investigations mentioned in the literature concerning the influence of hypothalamic lesions on the ACTH secretion are still rather conflicting. This may be partly due to the use of different criteria for ACTH release and to the use of different animal species. Besides, details on the site and extent of the lesions are often failing.

Several authors reported that lesions destroying the posterior hypothalamus abolish the ACTH secretion following a stressful stimulus. DE GROOT & HARRIS (1950, 1952) found that destruction of the posterior part of the tuber cinereum and mamillary bodies inhibited the lymphopenic response to emotional stress (immobilization or subcutaneous faradism) in rabbits. 'Remote control' stimulation of these areas caused a lymphopenia. PORTER (1953, 1954) observed that lesions destroying the mamillary bodies and the posterior portion of the tuberal nuclei abolished the eosinopenic response to adrenaline, formalin and histamine; lesions in the anterior part of the hypothalamus were not effective in this respect. Electrical stimulation of the tuberal and the posterior regions induced a pronounced eosinopenia. SLUSHER & ROBERTS (1956) found that lesions in the tuber cinereum and anterior border of the mamillary bodies in rats blocked the adrenal ascorbic acid response to laparotomy. They claimed that these lesions would not have destroyed the median eminence, but the daily water intake was reported to be as high as 135 ml, which suggests a severance of the supraoptico-hypophysial tract. In the rat it is very difficult to destroy the tuber cinereum without interfering with the hypophysial stalk. ENDRÖCZI and MESS (1956) measured the lymphopenic response to adrenaline and the adrenal ascorbic acid depletion following unilateral adrenalectomy, combined with the injection of adrenaline, in rats bearing lesions

in the anterior hypothalamus and in the posterior region, destroying a part of the median eminence, the tuber cinereum and the ventral part of the mamillary bodies. The lymphopenic as well as the ascorbic acid response were abolished by the caudal lesions, which are in fact mainly tuberal. Stimulation of the tuber cinereum and the mamillary bodies during 5 minutes induced a significant ascorbic acid depletion. This depletion is inhibited by barbiturate anesthesia, but not by adrenal demedullation (ENDRÖCZI *et al.* 1956). In this laboratory no inhibiting effect could be observed from small lesions destroying several parts of the posterior hypothalamus, including the mamillary bodies, on the adrenal ascorbic acid response to unilateral adrenalectomy in rats (BOUMAN *et al.* 1957).

HUME (1949), working with dogs, was the first to find lesions in the anterior hypothalamus effective in abolishing the ACTH secretion following stress. Destruction of the supraoptic nuclei had no effect, but bilateral paramedian lesions in the anterior and middle area inhibited the eosinopenic response to adrenaline and insulin. Remote control stimulation, particularly of the anterior region, induced an eosinopenia (HUME & WITTENSTEIN 1950). More recently, GANONG & HUME (1954) showed that after unilateral adrenalectomy no compensatory hypertrophy of the remaining adrenal gland occurs when more than 50% of the median eminence is destroyed.

MCCANN (1953) found in rats that neither anterior nor posterior lesions abolish the adrenal ascorbic acid response to unilateral adrenalectomy and the eosinopenic response to adrenaline. Only if more than 80% of the median eminence is destroyed, no adrenal activation is possible. MCCANN interpreted these lesions as interfering with the discharge of vasopressin, and found a correlation between the effectiveness of the lesion and the daily water intake (MCCANN & BROBECK 1954). In cats, LAQUEUR *et al.* (1955) produced hypothalamic lesions destroying the anterior, tuberal (including the median eminence) or posterior (mamillary bodies) regions. The tuberal lesions prevented the eosinopenic response to adrenaline and laparotomy, while the adrenal steroid output was diminished. The other lesions had little effect.

In our experience only lesions destroying the median eminence were effective (BOUMAN *et al.* 1957), but if the dorsally adjacent area (ventromedial and dorsomedial nuclei) are also damaged, the effectiveness is enhanced. SCHMIDT *et al.* (1957) found that lesions destroying the ventromedial and dorsomedial nuclei in guinea-pigs inhibited the ACTH-induced adrenal necrosis after injection of diphthery-toxin.

In summary, it is generally accepted at the present that destruction of the

tuberal region, including the median eminence, inhibits the reflex ACTH secretion to stress. Several lesions claimed to be situated in the anterior or posterior region appear to have affected also parts of the tuberal zone. Lesions destroying the mamillary bodies are repeatedly reported to inhibit the lymphopenic or eosinopenic response, but not the adrenal ascorbic acid response. The reliability of the eosinophil test is doubted, for eosinopenia may occur in the absence of adrenal glands (SWINGLE *et al.* 1954, ESSELLIER *et al.* 1957) and, moreover, a lipide extract from posterior hypothalamus induces an eosinopenia in hypophysectomized and adrenalectomized rats (DEWIED, unpublished observations). On the other hand, as stimulation of the posterior hypothalamus results in an eosinopenia, lymphopenia and an adrenal ascorbic acid depletion, the cooperation of this area in stress reactions cannot be excluded. It is known that this stimulation causes an adrenal-medullary discharge of adrenaline, which hormone causes an ACTH secretion already in minute quantities.

2. MATERIAL AND METHODS

In all experiments female albino rats of an inbred strain (*Unilever*) were used, weighing 160—180 gm.

Lesioned animals. Hypothalamic lesions were made with the aid of a stereotaxic instrument, as described by GREER *et al.* (1955). The technique has been published in detail by BOUMAN *et al.* (1957), but the main points will be summarized below.

The rats were anesthetized with 3.25 mg Nembutal per 100 gm body-weight, injected intravenously into one of the lateral tail veins, except for the animals submitted to lesions in the posterior hypothalamus which were anesthetized with ether.

The animal is then placed in the instrument, and the head is fixed by adjustable ear bars and a bridle behind the teeth (*Plate II*, Fig. 1). By a midline incision the skull is exposed and the head is centered on the zero position of the instrument with the electrode just above the sagittal suture. The electrode is then brought into the required position for reaching the area to be coagulated, by setting the coordinates of the instrument, and holes are drilled in the skull with a small dental drill in order to permit the electrode to enter the brain. Then the electrode is lowered to the desired depth and the lesion is produced with a high frequency current of 4 secs. duration, increasing in intensity during the coagulation.

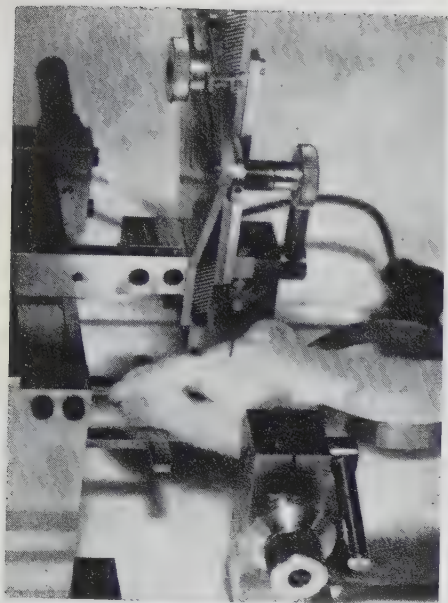
The location and extent of the lesions had been determined empirically beforehand. Bilateral lesions were made in the anterior, tuberal and poster-

PLATE II

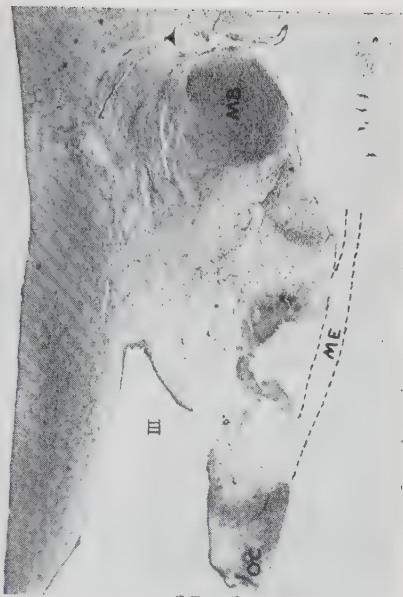
- Fig. 1. Experimental rat fixed in stereotaxic instrument for lesion procedure.
- Fig. 2. Sagittal section through the rat's hypothalamus, illustrating hypothalamic lesion destroying the tuberal area (Azan, x 17).

- Fig. 3. Sagittal section illustrating destruction of posterior part of hypothalamus (Azan, x 17).
- Fig. 4. Sagittal section illustrating destruction of anterior part of hypothalamus (Azan, x 17).

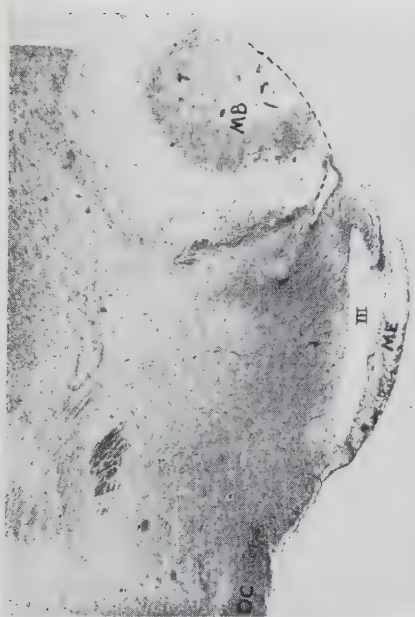
OC — optic chiasm
ME — median eminence
MB — mamillary body



1



2



3



4

ior part of the hypothalamus, at an angle of 5° from both sides in order to avoid haemorrhages from damage of the superior sagittal sinus.

The lesions in the anterior hypothalamus were produced by locating the tip of the electrode 1 mm above the base of the skull and at a distance of 0.6 mm from the median plane. This lesion did not affect the median eminence. The location of the posterior lesions was also 0.6 mm from the median plane, and just above the ventral surface of the mamillary bodies, in order to obtain a destruction of the ventral part of the posterior hypothalamus, but not of the hypophysis which is situated just under this region. Lesions in the tuberal region of the hypothalamus were made bilaterally, but in the median plane, with the tip of the electrode at the base of the skull, in order to destroy the median eminence and the dorsally adjacent area. For an illustration of these three lesions see *Plate II*, Fig. 2—4.

Nursing. The lesioned animals were permitted to recover for three days. Animals bearing lesions in the anterior or tuberal region, although more savage than normal, did not need any special care, but the animals with posterior lesions became hypothermic and lethargic (although they were easily aroused by minor stimuli as handling). They were therefore placed under a heating bulb and fed artificially. The mortality rate in these animals originally amounted to approximately 30%, but was reduced to $\pm 10\%$ by improvement of the diet and careful nursing; in the other lesioned groups the mortality rate was very low ($< 10\%$).

Hypophysectomized rats. In some experiments hypophysectomized animals were used. Hypophysectomy was performed by the parapharyngeal approach one day previously.

Control rats. The usual procedure of comparing operated experimental animals with sham-operated controls met with some difficulties in our experiments. The initial adrenal ascorbic acid content in sham-operated rats becomes very low, due to the permanent stress of the trauma, whereas in lesioned rats the ascorbic acid level is higher than normal, because the lesion destroyed hypothalamic areas involved in the hypophysial functions. Thus it was felt that sham-operated and lesioned rats are heterogeneous, and consequently not quite comparable.

The reason for using sham-operated animals generally is, that interference from the operational procedure with the observed phenomena is excluded. Therefore in additional experiments (see p. 66, Note) it has been shown that the lesion procedure in itself does not interfere with the adrenal response to stressful stimuli.

Thus it was decided to use normal intact rats as controls. These animals show an ascorbic acid level comparable with that in lesioned rats, provided that care was taken to avoid emotional stimuli which might affect the ascorbic acid level prematurely. The effect of these stimuli was also reduced by adapting them to handling and/or injections during several days.

Applied stressor stimuli. On the fourth day after the coagulation the animals were subjected to a stressful stimulus. The following stresses, including traumatic, painful, systemic and emotional stimuli, were used: unilateral adrenalectomy, adrenaline, histamine, formalin, Pitressin (Parke, Davis) electrical faradization, asphyxia, and strange environment combined with intense sound. In one experiment ACTH (Organon) was given as a direct stimulus to the adrenal cortex.

Unilateral adrenalectomy was performed under ether anesthesia. Mild faradic current was applied by placing the animals for 5 minutes in a cage with an electrified grid floor, which was connected with a RUHMKORFF coil. Asphyxia was given by tightening the scruff of the neck and closing the trachea with the thumb during 30 seconds. Sound was produced for 5 secs. every 30 secs. during 45 minutes by a buzzer, while the animals were placed in an aquarium with white painted bottom and sides, and a 100 W bulb placed just above the aquarium.

The following substances were injected: 30 γ adrenaline intraperitoneally, 2 mg histamine phosphate intraperitoneally, 10 γ ACTH intravenously, 0.2 I.U. Pitressin intravenously, all amounts per 100 gm body weight, in a volume of 0.5 ml.

Control animals received 0.5 ml saline intraperitoneally, if the stressor agent was a substance, or in the case of the other stresses, the mere handling belonging to the treatment, i.e. without electrical current or actual closing of the trachea. In the case of sound combined with strange environment the control procedure was restricted to handling and replacement into the cage. In the unilaterally adrenalectomized animals the removed left adrenal gland yielded the control ascorbic acid values.

Experimental procedures. The effect of every stress stimulus mentioned above was measured in rats bearing lesions in the anterior, posterior and tuberal hypothalamus, and in intact animals, on the adrenal ascorbic acid depletion according to the SAYERS test. In all cases except for the unilateral adrenalectomy, the MUNSON modification was used. One hour after the application of the stress the animals were sacrificed by decapitation and both adrenals were removed. The ascorbic acid determination was performed according to ROE & KÜTHER (1943) as modified by SAYERS *et al.* (1948).

Statistical analysis. In most cases the experiments had to be repeated in order to obtain sufficient data. The identically performed experiments were pooled, and the standard error of the mean (S.E.M.) was computed from the formula

$$\sqrt{\frac{\Sigma (\bar{x}_1 - x_1)^2 + \Sigma (\bar{x}_2 + x_2)^2 + \dots}{(n_1 + n_2 + \dots)(n_1 + n_2 + \dots - N)}}, \text{ in which}$$

$x_1, x_2, \dots$ = individual values in experiment 1, 2, ...

$n_1, n_2, \dots$ = number of values in experiment 1, 2, ...

N = number of experiments.

The statistical evidence for a difference between two experiments or two sets of experiments was computed according to Wilcoxon's two-sample test (WILCOXON 1945), from

$$t = \frac{|\Sigma U - \Sigma \mu| - 1/2}{\sqrt{\Sigma \text{var}}}, \text{ in which}$$

U = number of inversions

$\mu = 1/2 n_1 n_2$

$\text{var} = \sigma^2 U = 1/12 n_1 n_2 (n_1 + n_2 + 1).$

A difference was considered to be statistically significant if $t > 2.0$, or the double tail probability $p \leq 0.05$.

Histological methods. In every experiment with lesioned rats 5 animals were chosen at random, and their hypothalamus was examined histologically for the exact site and extent of the lesion. In all other lesioned animals the location of the lesions was examined macroscopically. In the exceptional cases that the lesions were located in a wrong place the animals concerned were discarded. For histological procedures a block containing the lesioned area and surrounding tissue was cut out, fixed in Susa and embedded in paraffin. Serial sections were cut at 10μ and stained with an azan modification.

3. RESULTS

A. The effect of several stresses in normal and lesioned animals

Unilateral adrenalectomy (table 2). The tuberal lesions entirely prevented the ascorbic acid depletion occurring in normal rats after unilateral adrenalectomy under ether anesthesia. The adrenal response is partly inhibited in anterior lesioned rats; in contrast, in the posterior lesioned

group the adrenal activation is greatly increased. The ascorbic acid values of the right adrenal in both the anterior and posterior lesioned group differ significantly from that of the intact rats ($p < 0.05$).

TABLE 2
The effect of unilateral adrenalectomy in intact and lesioned rats.

Experimental Group	Ascorbic acid content of		% change	No. of Rats
	Left adrenal	Right adrenal		
Intact	439 $\pm$ 16.6 ¹⁾	283 $\pm$ 10.1	— 36	15
Tuberal Lesion . .	461 $\pm$ 15.8	482 $\pm$ 16.9	+ 5	14
Anterior Lesion . .	473 $\pm$ 20.8	370 $\pm$ 27.8	— 22	13
Posterior Lesion . .	424 $\pm$ 16.9	206 $\pm$ 14.5	— 51	14

¹⁾ Mean value in mg% $\pm$ S.E.M.

Histamine (table 3). In tuberal lesioned rats the effect of histamine was not abolished in three consecutive experiments; the drug still induced a statistically significant adrenal ascorbic acid decrease ($p < 0.01$). In contrast, the anterior lesion reduced the effect of histamine almost completely; the ascorbic acid depletion did not differ significantly from that in control animals ($p = 0.16$). The posterior lesion caused a marked increase in adrenal response to histamine.

Adrenaline (table 3). The ascorbic acid depletion after injection of adrenaline in tuberal lesioned animals is probably abolished, for there is no significant difference with the control values ($p = 0.54$). The anterior lesion partly inhibited the adrenaline effect, whereas the posterior lesion did not influence the adrenal response significantly.

TABLE 3
The effect of histamine and adrenaline in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after				
	Saline	Histamine	% change	Adrenaline	% change
Intact	404 $\pm$ 14.6 (18)	303 $\pm$ 21.2 (18)	— 25	312 $\pm$ 10.4 (18)	— 23
Tuberal Lesion . .	439 $\pm$ 9.1 (16)	363 $\pm$ 11.0 (15)	— 18	413 $\pm$ 13.4 (17)	— 6
Anterior Lesion . .	415 $\pm$ 11.8 (15)	386 $\pm$ 18.4 (18)	— 7	354 $\pm$ 22.3 (18)	— 14
Posterior Lesion . .	424 $\pm$ 23.0 (12)	267 $\pm$ 12.8 (12)	— 37	341 $\pm$ 12.4 (13)	— 20

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

Faradic irritation (table 4). The effect of irritating faradic current is abolished in tuberal lesioned rats. Both the anterior and the posterior lesions diminished the effect to the same extent.

TABLE 4
The effect of irritating faradic current in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after		% change
	Handling	Faradization	
Intact	460 ± 10.4 (16)	283 ± 4.1 (17)	— 38
Tuberal Lesion	444 ± 7.5 (16)	450 ± 9.2 (16)	+ 1
Anterior Lesion	432 ± 11.9 (16)	356 ± 13.2 (17)	— 18
Posterior Lesion	418 ± 15.0 (15)	341 ± 11.1 (15)	— 18

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

Formalin (table 5). No adrenal response to formalin was observed in tuberal lesioned rats. The anterior lesion reduced the effect of formalin ($p = 0.05$). In posterior-lesioned animals the effect remained unchanged.

TABLE 5
The effect of formalin stress in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after		% change
	Saline	Formalin	
Intact	454 ± 13.2 (10)	280 ± 7.7 (10)	— 38
Tuberal Lesion	481 ± 17.0 (10)	494 ± 11.3 (10)	+ 3
Anterior Lesion	422 ± 26.2 (8)	310 ± 24.9 (9)	— 26
Posterior Lesion	434 ± 26.1 (10)	253 ± 22.2 (10)	— 42

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

Asphyxia (table 6). The tuberal lesions inhibited the ascorbic acid depletion following the application of asphyxia. The anterior and posterior lesions both diminished the effect of asphyxia significantly.

TABLE 6

The effect of asphyxia in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after		% change
	Handling	Asphyxia	
Intact	432 ± 13.4 (9)	269 ± 8.4 (10)	— 38
Tuberal Lesion	574 ± 22.2 (8)	552 ± 17.2 (8)	— 4
Anterior Lesion	451 ± 25.0 (10)	349 ± 21.1 (11)	— 23
Posterior Lesion	464 ± 21.8 (12)	336 ± 12.9 (13)	— 28

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

Sound and strange environment (table 7). The tuberal as well as the anterior and the posterior lesions completely inhibited the adrenal activation to the stress of emotional excitement by strange environment combined with sound.

TABLE 7

The effect of strange environment combined with sound in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after		% change
	Handling	Strange environ- ment + sound	
Intact	446 ± 22.8 (11)	309 ± 8.8 (11)	— 31
Tuberal Lesion	470 ± 15.0 (9)	457 ± 20.6 (10)	— 3
Anterior Lesion	406 ± 21.4 (11)	403 ± 16.5 (11)	+ 1
Posterior Lesion	437 ± 20.9 (10)	464 ± 20.6 (9)	+ 3

¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

B. *The effect of direct hypophysial and adrenal activation in normal and lesioned rats*

ACTH (table 8). The effect of ACTH in hypophysectomized, anterior and posterior lesioned rats on the adrenal ascorbic acid depletion appeared to be equal.

TABLE 8

The effect of ACTH in hypophysectomized and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after	
	Saline	10 γ ACTH
Hypophysectomized . . .	421 $\pm$ 21.1 (5)	295 $\pm$ 11.2 (5)
Anterior Lesion	398 $\pm$ 33.6 (7)	293 $\pm$ 7.8 (7)
Posterior Lesion	404 $\pm$ 23.1 (7)	297 $\pm$ 20.8 (7)

- ¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

Pitressin (table 9). In tuberal lesioned rats the effect of *Pitressin*, which is supposed to contain the CRF, is about the same as in normal animals. In anterior and posterior lesioned animals the adrenal response is perhaps somewhat diminished, although the difference between these groups and the intact group is not significant.

TABLE 9

The effect of *Pitressin* in intact and lesioned rats.

Experimental Group	Adrenal ascorbic acid content ¹⁾ one hour after		% change
	Saline	<i>Pitressin</i>	
Intact	407 $\pm$ 13.9 (9)	297 $\pm$ 5.6 (9)	— 27
Tuberal Lesion	443 $\pm$ 13.1 (11)	331 $\pm$ 12.8 (11)	— 25
Anterior Lesion	414 $\pm$ 16.8 (10)	336 $\pm$ 23.5 (13)	— 19
Posterior Lesion	420 $\pm$ 23.0 (12)	332 $\pm$ 24.9 (10)	— 21

- ¹⁾ The mean values are given in mg% with their S.E.M.; between brackets the number of animals is indicated.

The results are summarized in table 10. As the mean control values of the individual groups show some variation, the ascorbic acid depletion is given in percentages of the control values. Moreover, in order to compare the adrenal response in the lesioned groups is also given as a percentage of the depletion in intact animals.

TABLE 10

The effect of several stresses in normal and lesioned rats.

Types of Stress	% Change in Adrenal AA Content following Stress				Decrease in Adrenal AA Content in Lesioned Rats as % of Decrease in Normal Rats			
	Norm.	Tub.	Ant.	Post.	Norm.	Tub.	Ant.	Post.
unilateral adrenalectomy	— 36	+ 5*	— 22	— 51	100	— 14	64	159
histamine . .	— 25	— 18	— 7*	— 37	100	61	28	149
adrenaline . .	— 23	— 6*	— 14	— 20*	100	26	61	87
faradization .	— 38	+ 1*	— 18	— 18	100	— 3	53	53
formalin . .	— 38	+ 3*	— 26	— 42*	100	— 8	68	110
asphyxia . .	— 38	— 4*	— 23	— 28	100	11	60	74
strange environment								
with sound .	— 31	— 3*	+ 1*	+ 3*	100	10	— 3	— 10
Pitressin . .	— 27	— 25*	— 19*	— 21*	100	93	70	78

* Difference with *normal* values not statistically significant.• Difference with *control* values not statistically significant.

4. DISCUSSION

In these experiments the influence of several lesions on the adrenal response to several stressor stimuli was compared. As these experiments were not performed simultaneously, casual differences caused by unknown influences (like season, temperature, subjective aptitude of the experimenter, etc.) might have obscured certain phenomena or induced false results. In order to meet these difficulties, most experiments were done two or three times. Moreover, the order in which the experiments were done, was rather fortuitous. Thus for instance an experiment with formalin in tuberal lesioned rats was actually followed by an experiment with asphyxia in posterior lesioned rats, which in turn preceded an experiment with faradization in normal animals. In this way it was unlikely that certain results were influenced by a not observed change in lesion technique or other technical methods.

In some cases several stressor stimuli were combined in one experiment. This occurred with adrenaline, histamine and Pitressin, which drugs were given simultaneously in three experiments (Pitressin in two of them).

In one case two different lesion groups were used together: the experiment with ACTH (table 8) was performed on one day.

The results show (table 10) that the tuberal lesion, destroying the median eminence and the dorsally adjacent area, inhibited the adrenal response to all stressor stimuli, except for Pitressin and histamine. The effect of Pitressin remained unchanged, which is in favour of the assumption that this drug contains a CRF. Moreover, the other lesions also did not alter the effect of Pitressin significantly. The effect of histamine is only partially inhibited by the tuberal lesion. This result is difficult to explain. One is inclined to conclude that histamine at least partly stimulates the adeno-hypophysis directly and this would be in accordance with the idea that histamine is identical with a CRF (HARRIS *et al.* 1952, SWINGLE *et al.* 1956). However, in rats bearing anterior lesions histamine was not able to cause a significant adrenal activation, whereas in posterior lesioned rats its activity was markedly enhanced. These facts show that the action of histamine is not independent of the hypothalamus.

A comparable situation might exist in the case of Pitressin, the CR-activity of which is claimed to be at least partly blocked by brain stem transection (SAYERS 1957), although it is known that it is not inhibited by tuberal lesions. If this were true, it might be possible that the transection of afferent hypothalamic nerves to the pars distalis by destruction of the median eminence affords the opportunity to these substances to exert a vasomotor effect on the glandular hypophysial tissue, resulting in an ACTH release, which does not occur in animals with an intact hypophysial innervation. It is possible that adrenaline in tuberal lesioned rats also still may exert some ACTH-releasing activity, as a complete inhibition could not be demonstrated with certainty in our experiments. Adrenaline, histamine and vasopressin are substances with a strong vasomotor activity, although not in the same direction.

Concerning the effect of the anterior and posterior lesions, the results indicate that they exert a remarkable modifying influence on the adrenal response to several stressor stimuli. This modifying influence can be supposed to occur at three levels:

a) *the adrenal level*, by an increase or decrease in the responsiveness of the adrenal cortex to ACTH, presumably via a modified autonomic nervous tone;

b) *the hypophysial level*, by an analogous change in responsiveness of the pituitary gland to the hypothalamic activation, probably occurring via a CRF;

c) *the hypothalamic level*, by a change in the rate of ACTH-releasing activity.

Therefore these possibilities were checked by a comparison of the adrenal sensitivity to exogenous ACTH and of the hypophysial sensitivity to Pitressin in rats bearing anterior or posterior lesions. The data show that there are no arguments in favour of an influence of these lesions on the adrenal or hypophysial responsiveness. It is assumed, therefore, that the modifying influences occur at the hypothalamic level, by a change in the rate of ACTH-releasing activity.

From Table 10 it is apparent that in lesioned animals the different effects on the hypophysial activity are not only dependent of the site of the lesion, but also of the type of stress. If we examine the nature of these stresses, it can be stated that they are composed of four different kinds of noxious stimuli, i.e.: traumatic stimuli (causing tissue damage), systemic stimuli (acting via the systemic blood circulation), painful and emotional stimuli (acting via sensoric pathways on the central nervous system). We propose to denote traumatic and systemic stimuli as *somatic*, painful and emotional stimuli as *psychic*.

It can be inferred from our data that by destruction of the posterior part of the hypothalamus the effect of unilateral adrenalectomy and of histamine is enhanced, that the effect of formalin and adrenaline is not altered, that the effect of faradization and of asphyxia is diminished, and that the effect of strange environment in combination with sound is entirely abolished.

Since the latter three types of stress, particularly the last one, have little or no somatic components, but are mainly psychic, the suggestion is that a posterior lesion blocks the effect of psychic stimuli. In contrast, the same lesion may enhance the effect of somatic stimuli, as the other stresses are for a great deal somatic.

The latter assumption might be checked by complete elimination of the psychic component in mainly somatic stresses by permanent anesthesia. However, this appeared to be impossible, since posterior lesioned rats are far more susceptible for barbiturates than normal and otherwise lesioned rats. As these rats require a smaller dosis of Nembutal than the other lesioned groups to obtain the same level of anesthesia, it would be possible that a more marked effect of the somatic stress in the posterior lesioned group had been due to the lower quantity of Nembutal.

Nevertheless, in stresses with mainly somatic components the ACTH release is always diminished in rats bearing anterior lesions, but unaffected or even enhanced in animals with posterior lesions. This means that the anterior region is somehow involved in the ACTH releasing mechanism, whereas the posterior part may have no direct role in the mechanism of somatic stress. Apparently its destruction causes a potentation of the stress effects in some cases. It has been tried to apply a psychic stress

without hardly any somatic component by putting the animals into a strange environment, and in addition exposed to continuously repeated noise. The effect of these psychic stimuli is completely abolished by anterior as well as by the posterior lesion¹). We conclude, therefore, that both areas are indispensable for the effect of painful and emotional stimuli.

If we assume that destruction of the posterior hypothalamus abolishes the effect of psychic stimuli, but enhances the effect of somatic stimuli, then it is obvious that the effect of a stress with equal somatic and psychic components remains unchanged in these animals. It is conceivable that a formalin stress fulfills these conditions. As this agent was given without anesthesia, its traumatic properties were matched by its painful consequences, and so the stimulating effect of the posterior lesion on the somatic component may have been antagonized by the inhibiting effect on the psychic component, which resulted in a neutralization of both effects. Adrenaline undoubtedly stands alone in the series of stressor agents, which will come up for discussion later on. Suffice it here to remark that, if it is true that adrenaline inhibits the posterior hypothalamus in normal animals (GELLHORN, cf. p. 36), then the adrenaline effect must be about the same as when the posterior area is inhibited mechanically.

Strictly spoken, the division in somatic and psychic stress is an artificial one, as it is impossible to apply a pure somatic or a pure psychic stress. But if we discern somatic and psychic *components* of a stress, then it is reasonable to expect that somatic stimuli and psychic stimuli act on the hypophysis

¹) As it appeared that the effect of psychic stress is abolished by all three lesions we placed, it can be argued that this might be an unspecific effect of the lesion procedure as such. Therefore it is relevant to state here that in additional experiments the influence of a sham-operation (consisting of exposure of the skull, drilling of a hole and insertion of the electrode into the dura) on the effect of a somatic and a psychic stress was investigated. The ascorbic acid depletion following unilateral adrenalectomy and following exposure to strange environment combined with noise in sham-operated animals is given below.

Unilateral adrenalectomy:

Left adrenal	318 ± 23.4 (8)	
Right adrenal	228 ± 10.9 (8)	p < 0.01

Strange environment:

Control rats	336 ± 15.5 (10)	
Stressed rats	274 ± 6.0 (10)	p < 0.01

It is concluded that in spite of the low initial ascorbic acid values, due to the presence of a trauma, a somatic as well as a psychic stress induces a statistically significant adrenal ascorbic acid depletion. Thus the lesion procedure does not prevent the adrenal activation by noxious stimuli.

in a different way. An emotional stimulus must be conducted to the hypothalamus via other pathways than a somatic stimulus. It has been repeatedly found that discrepancies exist between the mode of action of somatic and psychic stimuli. Several data are reported which suggest that "systemic stress" acts directly on the pituitary gland, whereas "neurogenic stress" acts via the central nervous system (e.g. FORTIER 1952, FORTIER *et al.* 1957). Although these data are incompatible with the experience that hypothalamic lesions abolish the hypophysial response to stress, they do suggest that differences of that kind exist. Accordingly, DEWIED & JINKS (1958) reported that chlorpromazine inhibits the reflex ADH release following a painful stimulus, but not following the administration of histamine and nicotine.

Several reviewed conflicting results concerning the effect of lesions in the posterior part of the hypothalamus (cf. p. 51) may be explained by our data. HUME and MCCANN with their associates never observed an inhibiting effect of lesions destroying this region on the ACTH releasing activity of laparotomy, adrenaline, histamine, etc. In contrast, the inhibition of the lymphopenic response by similar lesions, reported by HARRIS & DEGROOT (1950) was only found after application of an emotional stress. The statements by PORTER (1953, 1954) that the eosinopenic response to adrenaline, histamine and laparotomy in cats and monkeys is abolished by posterior lesions, are not corroborated by our data. Perhaps this discrepancy is due to species differences. Moreover, we discussed already earlier the usefulness of the eosinophil criterion, especially with regard to the posterior hypothalamus (p. 19, 53). That direct stimulation of the posterior hypothalamus results in an enhanced liberation of ACTH is compatible with our as well as the referred experiments. The question is not if an activation of the posterior hypothalamus induces ACTH secretion, but if every kind of stress activates the posterior hypothalamus. In our opinion this is only the case in psychic stress. This will be amplier discussed in the next chapter, in which it is tried to offer an appropriate interpretation of our data.

IV

INTERPRETATION AND CONCLUSIONS

*"I have milked a thousand cows, but the
cheese I made is of my own".*

Charles Reade

From our experiments with hypothalamic lesions we concluded that somatic and psychic stimuli both cause ACTH discharge, but that in this mechanism hypothalamic centers are involved in different ways. We shall try now to detect why these differences exist and how they are caused.

Somatic stress is not capable to induce an ACTH secretion in tuberal lesioned rats. It is accepted that by these lesions the elaboration of the CRF is abolished. The area responsible for the production and release of CRF may be the ventral middle hypothalamus. A lesion destroying the posterior part of the hypothalamus (in which the ergotropic system is mainly situated) causes a potentiation of the effect of somatic stimuli, whereas destruction of the anterior part of the hypothalamus, representing the trophotropic region, diminishes the ACTH output following somatic stress. Coagulation of the ergotropic area induces according to GELLHORN (cf. p. 35) a shift in the hypothalamic balance to the trophotropic side. When we assume that the trophotropic area exerts a stimulating effect on the CRF-release, then our results can be explained by reasoning that the destruction of the anterior region reduces this stimulating activity, whereas destruction of the posterior region enhances the excitability of the anterior region and thus reinforces the potentiating effect of the trophotropic system on the CRF-release.

It is true that an analogous reasoning could be made by assuming that the ergotropic area represents an inhibitive centre. In that case an anterior lesion would intensify the inhibiting influence, whereas a posterior lesion would abolish the inhibitive action on the CRF-release. But this explanation meets insuperable objections when applied to psychic stress, for a destruction of the posterior area induces an inhibition of the ACTH release following emotional stimuli. Moreover, the anterior lesion has an inhibiting effect in all kinds of stress.

What happens actually in the intact animal during somatic stress? It is likely that the whole hypothalamus activated, for signs of increased sympathetic as well as parasympathetic activity are known as a consequence

of stress. This activation includes the CRF-releasing structures, which are influenced in addition by the trophotropic region in positive direction. The general hypothalamic activation as a result of somatic stress must be mediated by pathways leading to the hypothalamus. Since it is known that high pontine transection abolishes the hypophyseal response to stress (cf. p. 23), it is obvious to assume that the brain stem contains a receptive area for noxious stimuli (probably situated in the ascending reticular formation), and that nervous pathways from the brain stem conduct the impulses to the infundibular region of the hypothalamus. This is compatible with current ideas on the stress mechanism. According to French workers the reticular formation is activated by irritation of the peripheral sympathetic nervous system (p. 26). MAGOUN (1958) claims that "excitation of the reticular system is the route by which stressful stimulation leads to hypothalamico-pituitary activity and consequent ACTH elaboration". HARRIS (1958) stated that "there is no reason to doubt the existence of rich fiber connections between the midbrain and the tuberal area of the hypothalamus...". Since destruction of the mamillary bodies and their environment does not impair the somatic stress response, these pathways must reach the tuberal region without running through the former area. Further information on such pathways is provided by the interesting anatomical considerations given by ANDERSON *et al.* (1957). They informed that from unpublished work of NAUTA & KUYPERS afferent fibres are known which originate from the dorsal region of the brain stem (the tegmental nuclei of Gudden) running rostrally over the mamillary system on the dorsal side and then coursing ventrally to terminate in the tuberal hypothalamic region. This tract is known as the ascending component of the bundle of Schutz or the dorsal longitudinal fasciculus. They conclude that the anatomical arrangement is such as to suggest that the midbrain tegmental nuclei serve as an activator for the hypothalamus via this fibre system (cf. Fig. 29 in their paper). These data provide a basis for the assumption, that the hypothalamus is activated by the brain stem without being inhibited by our posterior lesion.

We shall turn now to the mechanism of psychic stress. As the tuberal lesion blocks the effect of psychic stimuli as well, it obviously has the same final pathway as a somatic stress. Destruction of either the anterior or the posterior part of the hypothalamus abolishes the effect of a psychic stress. Thus under these conditions the assumption of modifying influences on the CRF release by these regions is unreasonable. It is more likely that these lesions interrupt pathways conducting the emotional stimuli.

As stated earlier (p. 34, 38), evidence exists for an "emotional circuit" (PAPEZ 1937) leading from the rhinencephalon towards the posterior hypo-

thalamus and upward again via the mamillo-thalamic tract. The limbic system projects to the hypothalamus via: 1. the medial forebrain bundle, which arises in the lower septal area and which is received by the posterior hypothalamic nucleus; 2. the stria terminals, which connects the amygdaloid nucleus with the anterior and tuberal region of the hypothalamus, and 3. the column of the fornix, originating from the hippocampus and mainly terminating in the mamillary bodies. As a psychic stress is first informed to the cerebral cortex by sensoric afferents, the stimulus has to be conducted from the cortex to the hypothalamus. It is very likely that the rhinencephalon is involved in this route.

As the mamillary area obviously is involved in psychic stress, one might speculate that for the ACTH secretion the fornical connections from hippocampus to mamillary bodies are of importance. This assumption is also supported by PAPEZ (1958), who remarks that "it seems obvious that hippocampal discharges are passed on to the hypothalamus and mamillary body. That such is the case in emotional disturbances evoked by various predicaments and exigencies of alarm may be assumed". As the column of the fornix is running through the anterior hypothalamus to the mamillary bodies, destruction of the mamillary region as well as of the anterior region would interrupt, therefore, pathways from the rhinencephalon which conduct the emotional stress stimulus to the hypothalamus, and subsequently abolish the hypophyseal stress response.

If this is true, the question arises as to the pathways leading from the mamillary bodies towards the hypophysis. It is unlikely that there are direct nervous connections. At any rate, such connections are unknown. Moreover, brain stem transection also abolishes the effect of neurogenic stress on the ACTH secretion, which is incompatible with this assumption. In our opinion there are two possibilities, which need not exclude each other:

- a. the stimulus activates the brain stem mechanism which represents the receptor area for noxious stimuli and then travels via the pathways which it has common with the somatic stress stimuli;
- b. the stimulus descends via the spinal cord and splanchnic nerves, and reaches the adrenal medulla, and the consequently released adrenaline stimulates the discharge of ACTH.

Concerning the first possibility, there is ample information that cortical and rhinencephalic stimulation results in an excitation of the reticular information (see DELL & BONVALLET 1956). Besides, it is known that fornical fibres arising in the septal region run via the mamillary bodies and the interpeduncular nuclei to the pontine tegmentum, probably

terminating in the tegmental nuclei of Gudden. ANDERSON *et al.* (1957) state that "the only known direct afferents to the tegmental nuclei of Gudden are from the limbic system" (cf. Fig. 28 in their paper). This arrangement is also found in the rat (NAUTA 1956). It may be not a coincidence that just from this region (the tegmentum of the brain stem) the dorsal longitudinal fasciculus originates which runs towards the tuberal area of the hypothalamus. It is conceivable that emotional stimuli travel via the fornix to the tegmental nuclei of the midbrain and then are conducted backward to the hypothalamus.

Also the second possibility requires some further consideration. It is known that stimulation of the posterior hypothalamus results in an adrenaline discharge from the adrenal medulla. It is also repeatedly stated that emotional stimuli are associated with an adrenomedullary hormone discharge (NEWTON *et al.* 1931; FUNKENSTEIN *et al.* 1952; FRANKSSON *et al.* 1954). The role played by adrenaline in the ACTH-releasing mechanism has been reviewed in our discussion of the theory of LONG. It is conceivable that this mechanism is of importance in the stress response, but only in the case of emotional stress. We discussed some observations which already suggested this possibility (p. 17—18).

Investigations on the effect of psychic stress in spinal sectioned rats are in progress, in order to examine this assumption. Preliminary results suggest that spinal transection indeed diminishes the adrenal response to a psychic stress stimulus, but not to a somatic stimulus. It is likely that endogenous adrenaline plays an important role in emotional stress as a mediator for ACTH release.

Detailed information on the mechanism of the adrenaline mediation is still lacking. Adrenaline may activate the same brain stem receptory area as other stressor agents do, since BONVALLET *et al.* (1954) showed that adrenaline produces an arousal reaction by acting directly upon the tegmental region of the brain stem. On the other hand, the results of SAYERS (1957) suggest that the site of action of the adrenomedullary hormones is the hypothalamus, since he found that their action on the ACTH release is not abolished by high brain stem transection. It is unlikely that adrenaline stimulates the hypophysis directly, as tuberal lesions diminish its effect. In connection with the interesting fact that a neurotropic stress depletes the ACTH content from the neurohypophysis (MIALHE-VOLOSS 1958), it is possible that adrenaline activates hypothalamic nervous pathways leading to the neurohypophysis.

We shall summarize now our conclusions and illustrate our view in a diagram (Fig. 6).

The ergotropic and trophotropic regions of the hypothalamus, inte-

grating and governing the autonomic nervous system, are both involved in the so-called stress mechanism. One of the numerous processes which they affect during stress, is the enhanced release of ACTH by the hypophysis. This is realized by influences upon the hypothalamic region which exerts ACTH-releasing activity (probably the infundibular region). The trophotropic area, situated in the rostral hypothalamic part, exerts a potentiating effect on this region, or is perhaps even a part of the CRF-elaborating system. The caudal part of the hypothalamus is not directly concerned with the CRF-release, but is part of the ergotropic system.

Somatic noxious stimuli ascend to the brain stem and cause an excitation of the reticular formation. The stimuli for the hypophysis are conducted to the hypothalamus via the ascending fibres of the bundle of Schütz, which terminates in the infundibular region elaborating the CRF.

Psychic stimuli descend from the cerebral cortex via the limbic system to the posterior hypothalamus; from this region they can be conducted to the tegmental brain stem area where they may be switched over to the ascending component of bundle of Schütz, which may conduct the impulses to the tuberal hypothalamus. Activation of the ergotropic system is also known to cause an adrenomedullary discharge of adrenaline, which hormone may be a mediator for the release of ACTH in emotional stress.

This schematic representation is of course oversimplified, but may serve as a hypothesis, which can explain our results and also several discrepancies in the current theories on the mechanism of ACTH release during stress. This may be clear from a comparison of our scheme with those of LONG, PORTER and SAYERS (Fig. 3, 4, and 5).

Figure 8

Proposed mechanism of hypophysial activation by somatic and psychic stress stimuli

ACH	=	adrenocortical hormones
ACTH	=	adrenocorticotropic hormone
ADR	=	adrenaline
AH	=	adenohypophysis
CC	=	corpus callosum
CRF	=	corticotrophin releasing factor
DLF	=	dorsal longitudinal fasciculus
Fx	=	column of fornix
MB	=	mamillary body
ME	=	median eminence
NH	=	neurohypophysis
OC	=	optic chiasm
SPL	=	splanchnic nerves
TEG	=	tegmentum

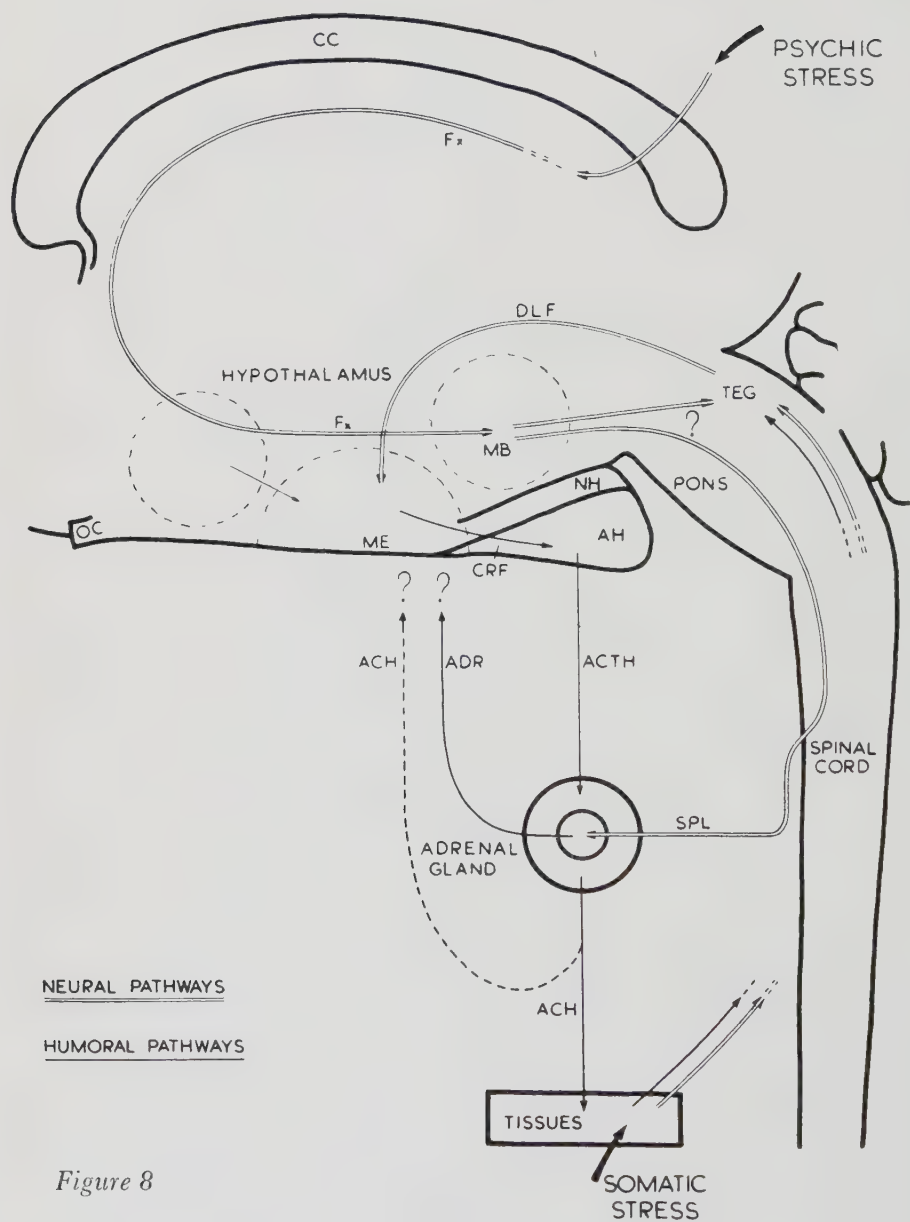


Figure 8

SUMMARY

The purpose of this study has been to investigate the influence of the autonomic nervous system on the secretion of the adrenocorticotrophic hormone (ACTH) from the pituitary gland following acute stress in rats.

First a survey is given of the endocrinological research on the stress mechanism (I, 2), in which special attention is paid to the stress concept of SELYE (I, 2, A), and the current views concerning the regulation of the ACTH secretion by the systemic blood level of the adrenocortical hormones (I, 2, B), by the adrenomedullary hormones (I, 2, C) and by humoral factors elaborated by the hypothalamus (I, 2, D). It is concluded that the experimental work in the endocrinological field indicates that the central nervous system, particularly the diencephalon, exerts a controlling function on the secretory activity of the hypophysis, but that the exact mechanism of this regulation is still poorly understood.

Then a review is presented of REILLY's concept of the "irritation syndrome", which is caused by noxious stimuli irritating the peripheral sympathetic nervous system (I, 3, A). The French work indicates that the sympathetic irritation is centrally induced, and suggests that the reticular structures in the brain stem are indispensable for these reactions (I, 3, B).

After a comparative discussion of the concepts of SELYE and REILLY (I, 4), more attention has been paid to the role of the central nervous system in stress reactions (I, 5). The main investigations on the diencephalic regulation of the vegetative, defensive and affective functions of the body (HESS), and on the effect of noxious stimuli and emotional disturbances on the hypothalamic balance (GELLHORN) have been reviewed. This work shows that the diencephalon represents the integrative and controlling structure for the homeostatic reactions to disturbance of the somatic and psychic equilibrium of the organism, which reactions are mainly mediated through the autonomic nervous system. The sympathetic and parasympathetic divisions of this system are represented in respectively the posterior and the anterior part of the hypothalamus.

Thus the influence of the autonomic nervous system on the ACTH secretion may occur at two levels: the peripheral level, through the peripheral autonomic innervation of the pituitary gland; and the central level, through a direct action of the diencephalic autonomic structures on the release of stimuli (nervous and/or humoral) descending via the hypophysial stalk (I, 6). Consequently, the experimental work is concerned with aspects of the peripheral and the central effects, i.e. with the influence of

faradic irritation of the sympathetic hypophyseal innervation on the ACTH release (II), and with the influence of hypothalamic lesions on the ACTH secretion following stress (III).

Some anatomical data on the innervation of the pituitary gland are provided in II, 1. Next the histological changes in the pars distalis of the hypophysis due to sympathetic irritation are described (II, 2, A). The ACTH releasing capacity of faradic irritation is measured in animals, in which a central activation is blocked by pretreatment with corticosteroids (II, 2, B). It is concluded that the tissular damage which results from sympathetic irritation and causes a direct contact between the glandular parenchyma and the blood stream, cannot explain the mechanism of the ACTH release (II, 3).

The experimental work on the regulation of the ACTH secretion with the aid of lesions placed in the hypothalamus has been discussed (III, 1). The present experiments in rats were concerned with a comparison of the effects of three different hypothalamic lesions on the ACTH secretion following the application of acute stress. These lesions destroyed the tuberal area (including the median eminence), the anterior ("trophotropic" or parasympathetic) part, or the posterior ("ergotropic" or sympathetic) part of the hypothalamus. The effect of traumatic, painful, systemic and emotional stimuli on the ACTH secretion was measured indirectly on the adrenal ascorbic acid depletion in these three groups of lesioned animals and in normal intact animals (III, 3).

It can be inferred from the results that the tuberal lesion abolished the effect of all types of stress, except for histamine. The anterior lesion diminished the effect of somatic (= traumatic and systemic) stimuli, and abolished the effect of psychic (= emotional and painful) stimuli. Psychic stimuli were also abolished by posterior lesions, but somatic stimuli were potentiated by these lesions. The effect of ACTH and Pitressin remained unaffected in all lesioned groups (III, 4).

A hypothesis is advanced concerning the mechanism of ACTH release in somatic and psychic stress. Somatic stimuli are supposed to activate the reticular formation of the brain stem, from where impulses pass on to the tuberal area of the hypothalamus, causing the release of the transmitter substance which activates the adeno-hypophysis for ACTH secretion. Psychic stimuli are thought to be conducted from the cerebral cortex via the limbic system and the descending column of the fornix to the posterior hypothalamus. Activation of this area may result in an activation of the reticular formation, and also in a stimulation of the adrenal medullary secretion, resulting in the release of adrenaline, which hormone on its turn causes an ACTH discharge (IV, see also Fig. 8).

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Microphotographs were made with great care by Dr. J. J. WACHTERS.

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STELLINGEN

I

De argumenten die FOLKOW aanvoert tegen het bestaan van een zelfstandig perifeer zenuwnetwerk, zijn niet overtuigend.

FOLKOW, B., *Physiol. Rev.* 35 (1955) : 629.

II

Het begrip „Leerlaufreaktion” (LORENZ) is een overbodige en verwarrende abstractie, en dient als zodanig te verdwijnen.

III

Het „Mittelrippenphänomen”, door STRUGGER in bladeren van *Helodea densa* gevonden, kan het beste verklaard worden door aan te nemen dat het hier een fysisch transport langs grensvlakken betreft.

IV

De inheemse *Cochlearia*-soorten *officinalis* en *anglica* dienen te worden teruggebracht tot één species.

V

De vergelijkende anatomie zal zich moeten verwijderen van de phylogenetische terminologie, en meer recht moeten doen aan de idee van de typologische structuur der taxa.

VI

De tot nu toe bekende factoren, die verantwoordelijk zouden zijn voor de evolutie der levende natuur (namelijk natuurlijke selectie, werkend op een patroon van kleine variaties, veroorzaakt door mutatie, bastaardering en geografische isolatie), zijn onvoldoende om een „macro-evolutie” aannemelijk te maken.

VII

De hypophysaire necrose, zoals beschreven in het syndroom van SHEEHAN, wordt niet veroorzaakt door thrombusvorming, maar door irritatie van de sympathische innervatie.

VIII

In de hypothalamus worden verschillende polypeptiden gevormd, die de afgifte van ACTH 'direct stimuleren.

IX

Een inleidend college in de natuurfilosofie is dringend gewenst voor alle gevorderde studenten in de natuurwetenschappen.

